1

Airway Smooth Muscle in Asthma Symptoms: Culprit but Maybe Innocent

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1. Introduction

The main function of smooth muscle found in either the airways or in other hollow organs is to contract. Once stimulated to contract, the smooth muscle strives to shorten. In turn, smooth muscle shortening narrows the lumen of the organ it surrounds. Contraction usually serves a physiological purpose, such as increasing arterial tension for vascular smooth muscle, micturition for the detrusor muscle or parturition for the uterus muscle. However, for the airway smooth muscle (ASM), the shortening narrows the airway lumen, which concomitantly increases the resistance to airflow. So it seems that every time ASM manifests its function it causes respiratory distress. This had raised the question of whether its existence is the problem (65, 162)?

One common respiratory disorder in which the symptoms are greatly engendered by ASM contraction is asthma. In fact, a proper asthma diagnosis involved testing the reversibility of airway obstruction with a bronchodilator, a drug relaxing ASM (usually a β_2-adrenoceptor agonist). A positive test is indicated by complete or partial reversibility, which simultaneously confirms the implication of ASM shortening in asthma symptoms. However, we know that a judge will never blame the gun for a murder. She/he would rather blame the assassin that pulled the trigger. Thus, the ASM could simply be an obeisant effector tissue that responds to external cues that are asking it to contract. So despite being culprit in the elaboration of asthma symptoms it may still be 'innocent'.

Hence, for asthma symptoms (at least the one mediated by airway narrowing) to be provoked, contractile stimuli need to be present. There is no doubt about the increased expression of spasmogens (i.e., contractile agonists) into asthmatic airways. Histamine (251), leukotrienes (125), endothelin-1 (148), prostaglandin D_2 (170), thromboxane A_2 (249), adenosine (61), bradykinin (136), anaphylatoxin C3a and C5a (121), substance P (231) and others, are all inflammatory mediators capable of stimulating ASM contraction and were all shown to be overexpressed in asthmatic lungs. These spasmogens are secreted/synthesized following exposure to environmental asthma triggers, such as allergens, viruses, bacteria, fungi, air pollutants, exercise, aspirin and/or cold dry air. The nature of the environmental trigger involved obviously varies among asthmatics but all of them ultimately lead to a type of airway inflammation with inflammation-derived spasmogens. However, not everyone who gets inflammation into her/his airways because of a cold, because they are exposed to

an allergen to which they are sensitized (atopic), because they do exercise, etc… gets asthma symptoms. So it might not be enough to have airway inflammation to get asthma symptoms. If you try to kill a moose with a pellet gun, you may have the gun and you may pull the trigger, but you are more likely to have no responsiveness. Saying that it may also be necessary to be responsive, or maybe hyperresponsive, to these inflammation-derived spasmogens to get asthma symptoms.

In fact, one of the pathognomonic feature of asthma is airway hyperresponsiveness (AHR). AHR is defined as an increased sensitivity and maximal narrowing in response to an inhalational challenge with a spasmogen (methacholine is the most commonly used). Whether AHR is a prerequisite to suffer from asthma or whether asthma is the cause of AHR is a contemporaneous debate and will be slightly addressed in this chapter. Some can argue that asthma can affect the degree of airway responsiveness and others would be just as right by arguing that AHR is a predisposing factor to be diagnosed with asthma. What is clear is that the degree of responsiveness is a good surrogate for the airway narrowing that will take place *in vivo* in response to endogenously produced spasmogens that are released either in normal state or during asthma exacerbation.

In a syndrome like asthma, understanding the factors involved in AHR may give important clues concerning the pathogenesis of asthma and the generation of asthmatic symptoms. As aforementioned, this is because the symptoms of asthma are caused, to a great extent, by airway narrowing induced by ASM shortening. Due to its unequivocal role in airway responsiveness, it is clear that the ASM plays an important role in AHR; without responsiveness there would be no hyperresponsiveness. However, whether the ASM is intrinsically different in asthma and responsible for AHR is still unclear. Several ASM dysfunctions, but also many other defects in non-muscle factors have been suggested to play a role in the manifestation of AHR. Whether these defects are genetically inherited or acquired as a result of disease processes is also a question of great interest. This chapter is an attempt to outline the current state of comprehension regarding the alterations in muscle and non-muscle factors that may contribute to the hyperresponsive phenotype seen in asthmatics.

2. Muscle factors

Studying ASM mechanics involves more than measuring its force-generating capacity. Many other ASM contractile properties may play a role in determining the degree of airway responsiveness *in vivo*, such as shortening amount and velocity, stiffness, ability to relax and to tolerate and/or recover from the decline in contractility induced by length perturbations. The term 'contractility' in this chapter is vague and refers to any contractile properties. So a hypercontractile ASM phenotype can mean one or all of the following: the muscle is stronger (increased force-generating capacity), it shortens more and/or shortens faster, it is stiffer, it has an attenuated ability to relax either spontaneously or in response to bronchodilators, or has an increased ability to tolerate and/or to recover from a drop in contractility caused by length perturbations. In the following section, these contractile properties are discussed individually and the rationale for their respective involvement in determining the degree of airway responsiveness *in vivo* is described. The published evidences suggesting that alterations to some of these contractile properties contribute to AHR in asthma are also briefly reviewed. The premise, here, is that AHR would be due to an inherited ASM hypercontractility; not one that would be acquired due to defects in non-muscle factors. Some of the factors discussed were addressed in a previous review (24).

It is worth-mentionning that the ASM has also been shown to proliferate, to migrate, to express adhesion molecules and receptors interacting with immune cells, as well as to synthesize extracellular matrix components, cytokines and chemokines. Most of these ASM functions were studied in monolayers of ASM cells in culture. More evidences are eagerly needed to confirm the existence of these ASM functions *in vivo*. However, if they happen *in vivo*, their relevance to asthma pathogenesis is unquestionable. These subjects have been reviewed lately and will not be addressed in the present chapter (18, 50, 72).

2.1 Force

The load impeding ASM shortening is auxotonic; i.e., it increases progressively as the muscle shortens. It is thus logical that greater force would lead to more shortening and concomitantly more airway narrowing. That is the reason why the force-generating capacity of ASM is such an important determinant of airway responsiveness.

The force-generating capacity also matters because it influences other ASM contractile properties. The relationship between the load and the velocity can be fitted with an exponential decay equation; so that increasing the load decreases the shortening velocity exponentially. This implies that a stronger muscle would counteract a given load faster and would thus shorten faster. In a context where the muscle is subjected to contract under a progressively increasing load, as it occurs *in vivo*, a stronger muscle would also shorten further. This is because a muscle able to produce more force at any given length would allow the shortening to progress further before reaching a load equal to its force. A stronger muscle would also increase ASM stiffness, which, as discussed below (subsection 2.2), can have an important impact on *in vivo* airway responsiveness.

The force or stress, which is the force per cross-sectional area, produced by the ASM depends on the potency and the concentration of the contractile stimulus involved. The relationship between spasmogen concentration and ASM-force can be described by a sigmoidal equation. So, *in vivo*, the amount of spasmogen reaching the ASM is one of the main determinants of the force produced by the muscle. The force produced by the ASM is also dictated by its length. Longer muscle generally generates more force in response to a given contractile stimulus (86, 154, 259, 261). In fact, the decrease in ASM-force caused by length reduction is proportional to he magnitude of the length change (103). Hence, *in situ* factors affecting the operating length of the ASM can be of considerable importance in the understanding of AHR, but that will be discussed later in this chapter (subsection 4.1.5).

Regardless of the aforementioned factors, the force can also be determined by the muscle's intrinsic capacity to generate force. So for a given concentration of a chosen spasmogen and a given length, the stress produced by the muscle can be different. This has led some to suggest that ASM derived from asthmatics may produce more stress than ASM derived from non-asthmatics, and that might be the cause of AHR. This hypothesis has been tested by several groups now and, although still debatable, the bulk of evidence suggests that the stress-generating capacity of asthmatic and non-asthmatic ASM is the same (reviewed in (153)). Taken together, the force-generating capacity of the ASM is certainly an important determinant of airway responsiveness, but no data published thus far convincingly demonstrate that this contractile property is altered in asthma.

2.2 Stiffness

In the field of ASM the term 'stiffness' certainly has different connotations. By definition stiffness is the amount of force required to cause a given change in length. The stiffness of

ASM can be either passive or active depending on whether the resistance to stretch stems from relaxed or activated components of ASM, respectively. Passive stiffness relies predominantly on the cell cytoskeleton. In fact, ablation of the cytoskeleton protein vimentin was shown to reduce passive stiffness by 3-fold (243). However, it came to experts' attention that the ASM is relatively compliant. The resting tension observed along the range of *in situ* operating lengths, even when the ASM is stretched to a level comparable to the one observed in a lung inflated to total lung capacity (TLC), is almost neglectable. On the other hand, the amount of tension generated by the same stretch in the presence of an active tone (i.e., in the presence of spasmogens) is disproportionally greater. For this reason, we focus here on ASM active stiffness, since it would be the principal component affecting airway responsiveness. Passive stiffness can also have a broader connotation if one considers the other components of the airway wall and the extracellular matrix (ECM) surrounding the ASM cells. These other passive elements obviously impact the overall stiffness of the airway wall and will be addressed later in this chapter (subsections 4.1.4 and 4.1.6).

Active stiffness is related to the level of ASM activation by spasmogenic stimuli. Its magnitude has always been thought to be dictated by the number of myosin heads bound to the actin filaments (i.e., number of cross-bridges). However, an emerging field in ASM suggests that other factors might play a part in active siffness. That is the level of interconnectivity between the ECM, the plasma membrane and the cell's cytoskeleton. These points of junction are excessively important for mechanotransduction efficiency; i.e., the transfer of individual resistive forces to the overall stiffness of the tissue during a stretch. Once considered as passive, recent studies rather suggested that the structures responsible for this interconnectivity can rearrange extensively upon ASM cell activation (reviewed in (264)). For example, Zhang and coworkers (263) have shown that α-actinin translocates to the plasmalemma and binds to β_1-integrin early after acetylcholine (ACh) activation, and blocking this interaction reduces active tension in response to ACh (263). This and other recent findings (105, 188, 265) confirmed that this interconnectivity between intracellular proteins, the plasma membrane proteins (integrins) and the ECM relies on dynamic processes that are activated by spasmogens. We shall henceforth consider this level of interconnectivity as an integral part of the active stiffness component of ASM.

ASM stiffness has captured the eyes of many scientists in the field recently because of its influence on airway responsiveness. To understand the link between stiffness and airway responsiveness, it is important to emphasize that ASM operates in a dynamic environment. The swings in transpulmonary pressure required for ventilation cause oscillating stresses on the airway wall, which, in turn, cause continuous variations of airway caliber. The effects of these oscillating strains (a strain is a change in length caused by a change in stress) on ASM mechanics are not small (Fredberg). In fact, in was predicted that the bronchodilating effect of oscillating strains at an amplitude that is thought to prevail *in vivo* due to tidal breathing is just as potent as high concentrations of a β_2-adrenoceptor agonist (isoproterenol) to reverse airway constriction induced by different contractile stimuli (84). In fact, a single stretch of the airway wall at greater amplitude seems to be sufficient. Stretching the airway wall by taking a deep inspiration (DI) has been recognized as a powerful way to induce bronchodilatation and bronchoprotection (171, 222). Inversely, refraining from taking regular DIs has been shown to increase AHR in normal (i.e., non-asthmatic) subjects (117, 118, 221). In the same vein, cessation of tidal breathing during breath-holding caused a decrease in tracheal and central bronchial diameter (165). This later observation suggests that the bronchodilating effect of breathing is omnipresent *in vivo,* and that removing the

oscillating airway wall strains caused by breathing allows the baseline level of ASM activation (tone) to constrict the airways. Finally, breathing at low lung volume has been shown to increase airway responsiveness (56), suggesting that not only the presence but also the amplitude of the airway wall strains induced by breathing impacts on the subsequent degree of airway narrowing provoked by a given spasmogenic challenge. Therefore, identifying the factors decreasing airway lumen expansion during a DI, or the factors limiting fluctuating strains of the airway wall during tidal breathing, such as passive and active ASM stiffness, are relevant to the understanding of airway narrowing and AHR.

The decrease in airway responsiveness induced by a DI has also been mimicked *ex vivo* on isolated porcine (178) and human (177) airways. In these studies, the liquid-filled airways were subjected to luminal volume changes reproducing the changes in transmural pressure occurring during tidal breathing (from 5 to 10 cmH_2O) and DIs (from 5 to 30 cmH_2O). The authors showed that the presence of DI simulations reduces the active pressure or the decrease in luminal volume produced by ASM in response to different concentrations of ACh.

Oscillations of airway caliber by breathing maneuvers seem to have a bronchodilating effect because they stretch the ASM. It was estimated that tidal breathing, sigh and DI, stretch the relaxed ASM by 4, 12 and 25% of its initial length, respectively (66). This is probably an overestimation since it was calculated based on changes in lung volume occurring during these breathing maneuvers while considering the lungs as isotropic material. Nevertheless, these length oscillations are known to decrease ASM stiffness, even during supra-maximal activation with ACh (66, 84, 128, 198). Importantly, the maximal force-generating capacity of ASM during or immediately following these oscillations is also reduced (66, 84, 85, 128, 186, 198, 242). The decline in isometric force that ensues length oscillations has been shown to be proportional to the amplitude and the duration of the stretch, but not to the frequency (242). These results demonstrated that the force produced by ASM in response to a given stimulus is greater in a static environment than a dynamic environment.

Oscillating strains at amplitude that is thought to prevail *in vivo* during breathing maneuvers also caused elongation of the contracted muscle (128, 130, 160). This later phenomenon is now referred to as force fluctuation-induced relengthening (FFIR). It also occurred in experimental settings more closely mimicking the *in situ* environment, where the ASM was subjected to an auxotonic load (186). Therefore, oscillating strains not only reduce the stiffness and the force-generating capacity of ASM in response to a given stimulus but also cause ASM relengthening. *In vivo*, this ASM relengthening will be translated into airway dilatation. The mechanisms underlying FFIR are not well understood but the length of the actin filaments seems to play a role (160).

Collectively, these studies have shown that the force (66, 84, 85, 128, 186, 198, 242) and the stiffness (66, 84, 128, 198) of ASM, as well as the length of the contracted ASM (128, 130, 160, 186), are affected by length oscillations (66, 84, 85, 128, 130, 160, 186, 198, 242) or simply by an acute stretch (242). Considering these phenomena together, one can envision the following *in vivo* vicious cycle. With exposure to spasmogens and the development of stiffer ASM, the same fluctuating stresses of breathing will cause less airway wall strains. This will allow the muscle to operate in a more static environment, where it will be able to produce more force and will be subjected to less FFIR. By producing more force, the muscle becomes stiffer, which further decreases the airway wall strains induced by breathing, and so on. Because the load impeding muscle shortening increases with the amount of airway narrowing, ASM shortening will eventually stop (when the force generated by the muscle is

equal to the load opposing its contraction). However, the repetitive sequence of events described above allows greater ASM shortening and consequently greater airway narrowing for any level of ASM activation. The link between stiffer ASM and AHR is thus indirect and explained by the fact that stiffening of ASM reduces the magnitude of airway wall strains (i.e., ASM stretches) caused by breathing maneuvers. In conjunction, these studies also suggest that the bronchodilating and bronchoprotective effect of breathing maneuvers seen *in vivo* may be due to he fact that ASM contractile properties are malleable and affected by straining forces.

Having said that, the bronchodilating effect of tidal breathing does not make unanimity (reviewed in (176)). In systems that more closely imitate the *in vivo* situation, such as in a liquid-filled airway segment subjected to transmural pressure oscillations mimicking the swings in transpulmonary pressure occurring *in vivo* due to breathing maneuvers, only the DI (but not tidal breathing) was shown to attenuate the increase in pressure (178) or the reduction in luminal volume (177) caused by ACh stimulation. Nevertheless, it will be important to determine whether the stiffness of the ASM is different between asthmatics and non-asthmatics. So far, the only evidence to support the conjecture that an increased ASM stiffness causes AHR comes from a study using animal cells. The ASM cells derived from the inherently hyperresponsive Fisher rats were shown to exhibit a higher stiffening response to a panel of spasmogens compared to the cells derived from the hyporesponsive Lewis rats (6).

2.3 Tolerance to oscillating stretches and rate of recovery following length perturbations

In the previous subsection, we have seen that length perturbations can greatly affect ASM contractility. From now on, by length perturbations we meant any of the following: a length change, either elongation or length reduction; a single stretch or release with an immediate return to the initial length; oscillating strains (length oscillations); or oscillating stresses (force oscillations) that is sufficient to modulate ASM length. The ability to tolerate and recover from these length perturbations are thus important ASM contractile properties that may influence the degree of airway responsiveness. The ASM's ability to maintain its force-generating capacity during shortening could also be important in determining the degree of airway narrowing. It is well-known that the ASM becomes 'weaker' as it shortens. So that the instantaneous capacity to produce force during (or immediately after a) length reduction is inversely proportional to the magnitude of the length change (103). This suggests that the force-generating capacity of ASM at shorter lengths is an important factor determining the extent of airway narrowing; simply because it dictates the remaining force available to counteract the loads which limit further airway narrowing at these new shorter lengths. There is currently no data comparing the decline in force caused by given reductions of ASM length between asthmatics and non-asthmatics.

Since we just came to realize the potentially important role of these contractile properties in airway responsiveness, it is not surprising that not enough comparisons were made between asthmatic and non-asthmatic tissues. The only evidence that we are aware of comes from our group (Leslie *et al*., accepted in the European Respiratory Journal). In that study, tracheal ASM strips derived from asthmatics and non-asthmatics were isolated and their ability to tolerate length perturbations and to recover from them was studied *ex vivo*. We found that the decline in force caused by length perturbations was attenuated in asthmatic tissues. The length perturbations used were a 60% length oscillations for 10 min, which are way beyond the length changes that would occur *in vivo*. The physiologic meaning of this finding may thus be

questioned. However, since all tissues were exposed to the same oscillating strains, it still means that there is an intrinsic difference between asthmatic and non-asthmatic ASM tissues in their ability to tolerate length oscillations. Interestingly, other ASM contractile properties were also compared in that study, such as the stress-generating capacity in response to electrical field stimulation (EFS), the velocity of shortening, the amount of shortening and the ability to relax. Among all the contractile properties tested, only the ability to tolerate length oscillations was clearly different between asthmatic and non-asthmatic ASM. These results suggested that the influence of disparate ASM in determining the different degree of airway responsiveness observed between asthmatics and non-asthmatics would only be manifested in certain circumstances… such as in a human that is breathing?? These results would need to be confirmed by other investigators.

The speed and the extent of force recovery following an initial decline in force induced by length perturbations could also contribute to the manifestation of AHR. In the aforementioned study using human tracheal ASM strips (Leslie *et al*., accepted in the European Respiratory Journal), no difference in the rate and extent of recovery was observed between asthmatic and non-asthmatic tissues. Since the decline in force induced by length oscillations was greater in non-asthmatic ASM, this implies that the force produced by the non-asthmatic ASM was lower during the entire recovery period (which was measured for 30 min). Also worth-mentioning is that this time period was sufficient for the asthmatic ASM to come back to its force before oscillations, which was not the case for non-asthmatic tissues. Other studies have used animal models to study the recovery of ASM-force following length oscillations. Wang and coworkers (241) measured the effect of length oscillations on isometric force-generating capacity of tracheal ASM strips from guinea pigs of different age groups. The force was assessed before and immediately after length oscillations, as well as at 6-min intervals thereafter to follow both the change and the recovery of force following oscillations. All age groups showed a similar decline in force immediately following length oscillations. However, whereas the force produced by tissues from older animals (3 week-old and adult) recovered to pre-oscillations levels over a time course of ~30 min, the force produced by the tissues from the youngest animals (1 week-old) rapidly rose above baseline (i.e., force before oscillations) and remained at this higher value for the entire time-window over which force recovery was measured. The increase in force over baseline induced by length oscillations was called 'force potentiation'. The molecular mechanisms underlying force potentiation are not well understood, but differential synthesis of prostaglandins seems to explain this age-dependent phenomenon in the guinea pig (44)).

A phenomenon closely related to force potentiation, which was dubbed the 'myogenic response', has also been suggested as a possible contributor to AHR in humans. Marthan and Woolcock (144) studied asthmatic patients in whom a DI induced a decrease in specific airway conductance. As discussed earlier, this paradoxical response is not uncommon in severe asthmatics and is an indicator of marked AHR (134). They found that nifedipine, a L-type calcium channel blocker, prevented the decrease in specific airway conductance induced by the DI (144). They suggested that the stretch of the ASM caused by the DI provoked a calcium-dependent bronchoconstriction (myogenic response).

Taken together, it seems clear that the tolerance to the decrease in contractility induced by length perturbations and the ability to recover from them may play a role in determining the degree of airway responsiveness. However, more data are warranted to confirm that these contractile properties can discriminate normo-responsiveness from AHR. Studying these contractile properties also unveiled other phenomena, such as force potentiation and the myogenic response, which can also be significant in the understanding of AHR.

2.4 Amount of shortening and velocity of shortening

The amount of ASM shortening is of major importance because it ultimately determines the amount of airway narrowing. As discussed earlier, the amount (as well as the velocity) of shortening depends on the ASM-force relative to the load. Therefore, all the factors influencing ASM-force, such as the potency and the concentration of the contractile stimulus involved, the quantity of spasmogens reaching the ASM, the muscle's intrinsic capacity to generate force, and its length, as well as all the factors influencing the load impeding muscle shortening affect the amount and velocity of shortening. However, the intrinsic ability to shorten may also be different between asthmatics and non-asthmatics. So that under the same load and despite producing the same stress, the amount of shortening achieved may be different. Interestingly, this has been shown in isolated ASM cells (140). In that study, the authors showed that unloaded ASM cells derived from asthmatics shorten more at room temperature in response to EFS. However, this observation, which now has a decade old, still awaits confirmation. The underlying mechanisms involved are also unclear but decreased resistance to shortening due to reduction in either internal resistive load (214, 226) or stiffness (44) has been proposed.

The shortening velocity of ASM could also be a critical determinant of the amount of airway narrowing. Again, to comprehend the potential implication of ASM shortening velocity in determining the degree of airway responsiveness, it is important to understand that ASM operates in a dynamic environment. The load impeding its shortening is continuously fluctuating due to swings in transpulmonary pressure caused by ventilatory maneuvers (e.g., tidal breathing and DI). The amount of ASM shortening *in vivo* is determined by a balance between the rate of cross-bridge cycling on the actin filaments causing muscle shortening versus the rate and the magnitude of stretch-induced disruption of cross-bridges causing muscle elongation (67). A faster cycling rate of the cross-bridges with a commensurate increased velocity of shortening would lead to more shortening during exhalation, when the load opposing muscle shortening is lowering. A faster rate of cycling would also lead to more cross-bridges being attached at the end of expiration, rendering the ASM and the entire airway wall stiffer. In turn, the stiffer airway wall would be less vulnerable to the stress imposed by the subsequent inspiration; i.e., the airway wall would be exposed to the same stress of breathing but the strain of the airway wall and, consequently, the stretch of the ASM would be attenuated in an airway with stiffer ASM. The combination of more shortening and more cross-bridge attachments during exhalation with less stretch and less cross-bridge detachments during inhalation means that the ASM with an increased shortening velocity would eventually reach a new equilibrium where the size of the airway lumen would be smaller than with a slower ASM. In addition, the airway wall that has reached this equilibrium becomes more static. Since ASM operating in a static environment produces more force in response to the same stimulus (as discussed above), it is possible that a faster muscle would not only cause more shortening and more cross-bridges during exhalation, but would also becomes stronger (i.e. able to produce more force for the same level of activation). By acquiring more force the ASM would then be able to narrow the airway further during the next exhalation and… the cycle can perpetuate itself. This vicious cycle is likely to happen in airways possessing ASM with faster shortening velocity and this is the rationale behind the idea that the speed of ASM contraction could be the cause of AHR.

Experimental evidences exist to support the hypothesis that a faster ASM velocity of shortening can contribute to AHR. The velocity of ASM shortening was shown to be greater in animal models in which there is innate AHR (reviewed in (132)). Similarly, there is

greater maximal shortening velocity in human ASM cells derived from asthmatics (140). Whether this increased velocity of shortening is innate or acquired due to asthma in humans remains to be determined. Two mechanisms have been suggested to be responsible for the observed increased velocity of shortening in asthmatic ASM: 1-The increased expression of myosin light chain kinase (MLCK) (3, 16, 133); and 2-a preponderant expression of the faster cycling smooth muscle myosin heavy chain (smMHC) isoform B over the slower cycling smMHC isoform A (reviewed in (132)).

MLCK is a enzyme capable of phosphorylating the regulatory myosin light chain (rMLC), which is a necessary step required for actin-activation of myosin ATPase activity and the subsequent binding and pivotal of the cross-bridges on the actin filaments. The rationale is that faster rMLC phosphorylation caused by the increased amount of MLCK would lead to the activation of more cross-bridges and a faster onset and velocity of shortening at early time-points following ASM stimulation. However, increased expression of MLCK in asthma is not a unanimous finding (147, 256). An alternate explanation for the increased velocity of shortening of asthmatic ASM is a differential expression of smMHC isoforms. The so called B isoform (also called the (+) insert isoform because of the presence of a 7-amino acid insert in the loop 1 of the protein) shows a greater rate of cross-bridge cycling *in vitro* (129). Its preponderant expression over the A isoform would likely increase the velocity of ASM shortening and, therefore, contributes to AHR. In accordance to this assertion, the ratio of the isoforms correlates with the level of airway responsiveness in rats; i.e., hyperresponsive animals expressed more of the B than the normo-responsive animals (73). The mRNA expression of the B isoform is also overexpressed in human asthmatics (133). Taken together, the amount and the velocity of shortening are potentially important factors determining the level of airway responsiveness. However, more data are needed to confirm that derangements in these contractile properties are involved in the manifestation of asthmatic AHR.

2.5 Ability to relax

ASM relaxation can also affect airway luminal diameter and the degree of airway responsiveness (reviewed in (69) and (44)). Just as stiffness, relaxation can have different connotations. It could refer to the relaxation either during or following the removal of the spasmogenic stimulation, as well as the relaxation induced by a relaxing agonist (bronchodilator). The time of onset, the rate and the extent of relaxation following stimulation with a relaxing agonist, or during or after the removal of the spasmogen, can also impact airway patency. The potential implication of impaired relaxation in asthma is clear. Incomplete or slower relaxation could keep the airways narrowed and, thus, prolong the respiratory distress experienced by asthmatics during an asthma attack. However, the mechanism by which impaired relaxation could contribute to AHR is not as obvious. It relies on the assumption that spontaneous relaxation occurs rapidly following airway narrowing induced by inhalational challenge with a spasmogen. Increased time for the muscle to relax following a spasmogenic stimulation certainly means that the airway luminal size will remain smaller for an extended period of time following a bronchoprovocative challenge and, consequently, will limit airflow during a forced expiratory maneuver (which is often used to assess airway responsiveness).

The following studies provide some evidences that impaired relaxation may play a role in determining the degree of airway responsiveness. Altered relaxation of ASM has been proposed to play a role in determining inherent differences in the degree of airway responsiveness observed between guinea pigs of different ages (reviewed in (44)). The absence or presence of spontaneous relaxation in response to continuous EFS has also been

proposed to be a factor explaining innate differences of airway responsiveness seen in different strains of mice (44). The role of impaired relaxation of ASM in AHR seen in human asthmatics needs further investigations.

To reiterate, contractility is defined here as a vague term that may imply different contractile properties. So a hypercontractile ASM phenotype may imply one or all of the following: the muscle may be stronger (increased force-generating capacity), it may shorten more and/or shorten faster, it may be stiffer, it may have an attenuated ability to relax either spontaneously or in response to bronchodilators, or it may have an increased ability to tolerate or recover from a drop in force caused by length perturbations. As seen in this last chapter's section, there are currently evidences suggesting that some intrinsic defects of ASM can contribute to asthmatic AHR. This also suggests that being hyperresponsive could be a prerequisite, or at least a predisposing factor, to be diagnosed with athma. However, whether these intrinsic defects are genetically inherited or acquired as a result of other defects seen in asthmatics is still unknown. One might expect that if asthmatic AHR is due to inherited ASM hypercontractility, it should be encrypted into the genome? The next section shall explore this question.

3. Interrogating the genome for asthma susceptibility genes

Genetics has evolved tremendously in the last decades and is now endowed with powerful tools to ask questions on the etiology of complex human traits (77). Asthma and the degree of airway responsiveness are irrefutably complex traits. Based on genetic epidemiology studies, the heritability of asthma was estimated to be 40 to 60% (22). However, finding genes and genetic variants responsible for this important genetic component is still very challenging. Not only asthma is a complex trait because of its heterogeneous nature, but also because its manifestation relies on the exposure of an (or many) environmental trigger(s). Thus, a lot of noise in genetic analyses of asthma may stand from the fact that some non-asthmatics (control cohort) are carriers of susceptibility alleles, but are not asthmatics because they never encountered the environmental trigger(s). In addition, maybe more than a single complex trait is required to develop clinical manifestations of asthma. For example, both AHR and airway inflammation may be required to suffer from asthma. If true, the noise in genetic analyses may arise from people with airway inflammation (e.g., atopy) that are protected from asthma symptoms because they are normo- or hypo-responsive. In fact, ~50% of the population is atopic but most of the affected individuals are non-asthmatics. On the other hand, some people may be carrier of alleles that confer susceptibility to AHR, which also confer susceptibility to asthma, but will always be free of asthma symptoms because they will never develop airway inflammation. In fact, it is estimated that roughly 15% of the population is hyperresponsive. Some of these people are asymptomatic, will never be diagnosed with asthma, and are counted in the control (non-asthmatic) group in most genetic studies despite being carriers of alleles conferring susceptibility to asthma.

Despite these challenges, recent progress in genetics of asthma was made by genome-wide association studies (GWAS). These studies consist of testing hundreds of thousands of single-nucleotide polymorphisms (SNPs) distributed across the human genome for association with a disease in hundreds or thousands of individuals. This genomic approach was particularly successful to identify robustly replicated genetic variants involved in complex diseases and biological traits (142). Asthma and asthma-related phenotypes are no exception and a number of GWAS were performed in the field (83, 104, 163, 164, 223).

Before the era of GWAS, more than 100 genes have been associated with asthma and related phenotypes (22, 183). Figure 1 shows these genes classified by their most likely pathobiological implication in asthma. Most of these genes were found using a candidate gene strategy (in blue), while fewer were found using genome-wide linkage studies (in green). Eleven genes were specifically studied for their possible involvement in bronchoconstriction and it is suspected that the risk conferred by genetic variants in these genes may act directly through ASM. However, for all these genes the functional demonstration remains to be made. In addition, candidate gene studies were plagued with

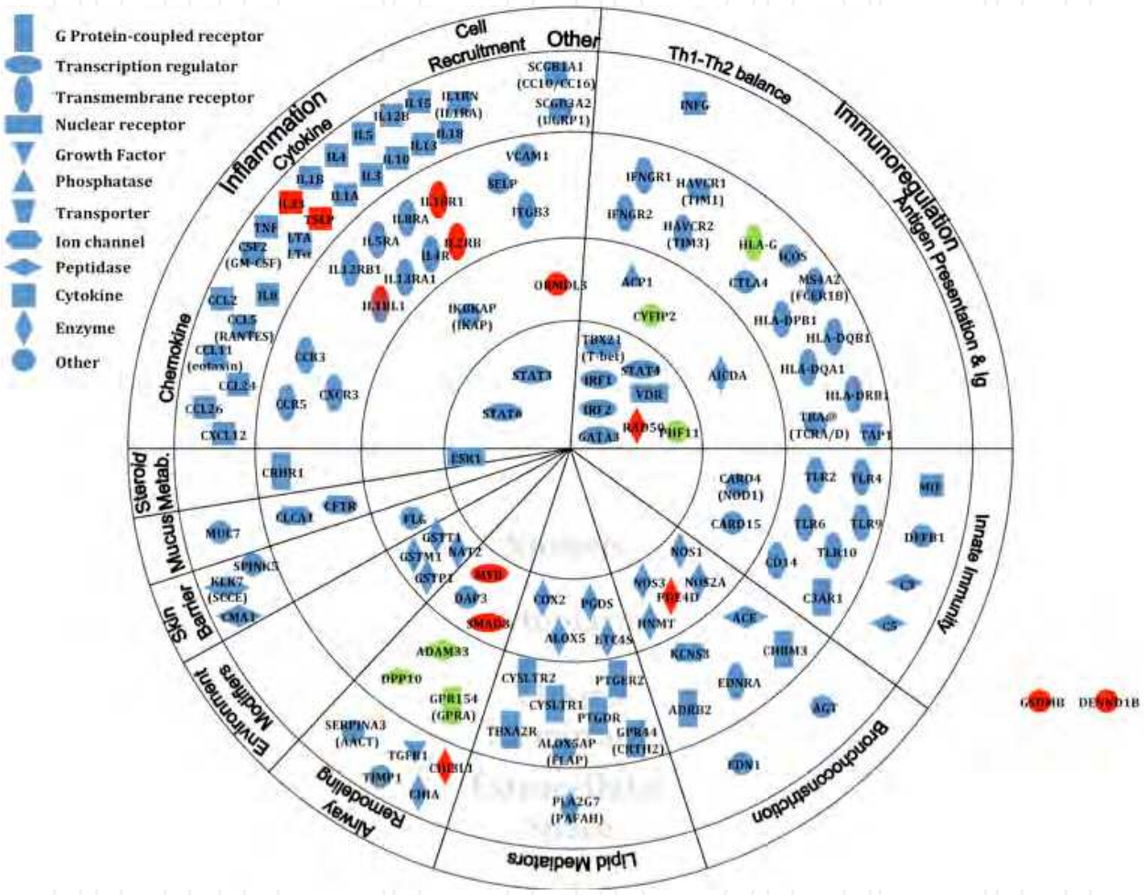

Fig. 1. Overview of asthma susceptibility genes. Genes illustrated are reported to be associated with asthma or asthma-related phenotypes in at least one published study. Each gene is categorized according to what we believe is its major role in the pathogenesis of asthma. In blue and green are genes identified using candidate gene and genome-wide linkage studies, respectively. The latest and more robust asthma susceptibility genes are illustrated in red and identified using a genome-wide association approach. Complete references for genes in blue and green can be found in Ober & Hoffjan (183). References for genes in red are in the text (see section 3). The shapes and subcellular localizations are taken from the Ingenuity System (http://www.ingenuity.com). Genes are labeled with official Entrez Gene symbols, and common alias names are shown in parentheses for some genes. The figure is updated from Bossé & Hudson (Annu Rev Med 2007; 58: 171-84).

inconsistency and genetic associations illustrated in Figure 1 require validation in large population samples.

In contrast to candidate gene studies, susceptibility genes derived from GWAS on asthma were confirmed in multiple and larger set of samples. These genes include ORMDL3, GSDMB, IL33, TSLP, IL18R1, IL1RL1, IL2RB, RAD50, MYB, SMAD3, CHI3L1, PDE4D, and DENND1B (illustrated in red in Figure 1). The specific cells and tissues that are molecularly altered by the risk variant in these genes are still unknown. Most of these genes are not known as regulators of airway responsiveness, suggesting that the genetic predisposition to suffer from asthma do not originate from genes altering ASM function. In fact, most of the GWAS-nominated asthma genes are expressed in the airway epithelium or in inflammatory cells, which is consistent with the growing body of evidences that asthma originates in subjects whose epithelium has altered response to environmental triggers and whose immune system is susceptible to the development of inflammation. The exception is the PDE4D gene found to be associated with asthma in multiple white and Hispanic populations (104). PDE4D is expressed in ASM and can potentially alter ASM contractile function, suggesting that this tissue, in addition to the epithelium and inflammatory cells, may influence susceptibility to asthma.

It should be noted that we cannot rule out the contribution of other GWAS-nominated genes acting on ASM. We know from our previous whole-genome gene expression experiment in bronchial smooth muscle cells (23) that approximately half of the genes in the human genome are expressed in non-stimulated ASM cells (nearly 10,000 genes). Obviously other genes might be inducible and expressed only in a 'sick' environment. However, at baseline, in a monolayer of ASM cells, many GWAS-nominated asthma genes are expressed including ORMDL3, GSDMB, IL33, TSLP, RAD50, SMAD3, CHI3L1, and DENND1B. Although what is known about the biological functions of these genes is not pointing toward ASM as the primary pathobiological target, their expression in this tissue suggests that they may play a role. The characterization of asthma susceptibility genes derived from GWAS is currently a priority in the field of genetics of asthma (182). The functions of these genes in ASM, if any, will need to be investigated, as are the consequences of the risk variants on these functions.

4. Non-muscle factors

In contrast to the above, AHR may also be secondary to the asthmatic syndrome. In fact, many causes of AHR in asthma are attributed to alterations in the lung environment; implying that the ASM can be absolutely normal but would lead to AHR upon activation because it operates in a 'bad' environment. Two scenarios here are envisaged: First, AHR may develop in asthmatic individuals because inflammatory or remodeling changes alter ASM contractile properties. In those instances, AHR still relies on a defect in ASM contractile properties, as discussed in section 2, but they are acquired as a result of an altered environment. Parenthetically, the phenomena that lead to an acquired increase in contractility will be called here an 'ASM behavior' (see Figure 2). Thus, a behavior is a normal ASM's ability to adapt to its surrounding environment. Some ASM behaviors are addressed in the non-muscle factors section of this chapter because their appearance is attributable to lung defects. This didactic distinction between ASM contractile properties and behaviors would become clearer along the remaining of this chapter. In the second scenario, certain causes of AHR are unrelated to genetically or acquired abnormalities of ASM but are rather due, exclusively, to lung alterations. In those instances, the abnormal milieu is sufficient to cause AHR in a setting

where none of the ASM contractile properties are altered. In the following section, we will describe the non-muscle factors that are potentially involved in asthmatic AHR by affecting, or not, the contractile properties of ASM. Four broad themes will be discussed; that is remodeling, airway inflammation, ASM-tone and ventilation heterogeneity. Some of the factors discussed below were addressed in previous reviews (24, 26).

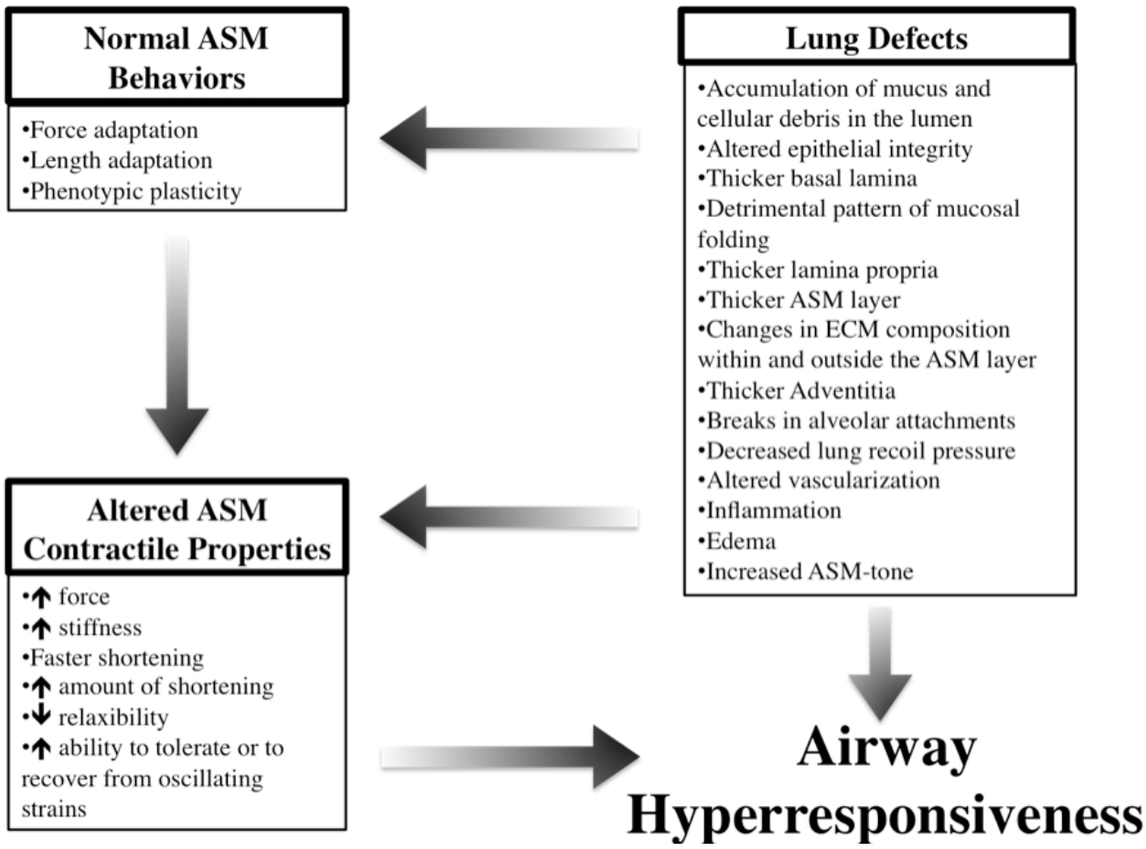

Fig. 2. Muscle and non-muscle mechanisms potentially involved in airway hyperresponsiveness (AHR) seen in asthmatic patients. Any alterations in airway smooth muscle (ASM) contractile properties, which can be innate or acquired as a result of lung defects, may participate in the development and manifestation of AHR. Lung defects can also contribute to AHR either directly or by fostering normal ASM behaviors, such as force adaptation. See text for further details.

4.1 Remodeling

The term 'remodeling' refers to any structural changes occurring in the lungs. It could be any alteration in the quantity, the distribution and the composition of the molecular, cellular and tissular constituents of the lungs. It can affect, or not, the physical and mechanical properties of the lung constituents. The term 'remodeling' is also usually reserved for structural changes that are permanent or fixed (relatively), such as the deposition of extra ECM components. So even if edema and inflammatory cell infiltrates fit into the definition of remodeling given above, these changes are not usually perceived as remodeling changes. They are rather the results of inflammatory processes that are transient and reversible in nature. Generally speaking,

remodeling can designate any or all of the following: epithelial metaplasia, such as zone of denudation, deciliated areas and goblet cell hyperplasia; fibrosis of all the airway wall layers but especially the *lamina reticularis* (the third layer of the basal lamina); vascular alterations, such as vessel enlargement and angiogenesis; thickening of all the layers of the airway wall including the epithelium, the basal lamina, the lamina propria, the ASM and the adventitia; and parenchymal changes, such as emphysema and breaks of alveolar attachments on the outer edge of the airway wall (Figure 3). Lung remodeling, especially airway remodeling, has been reviewed by many in the past (25). In the present chapter, we focus on the structural changes that have been shown or suggested to alter the level of airway responsiveness.

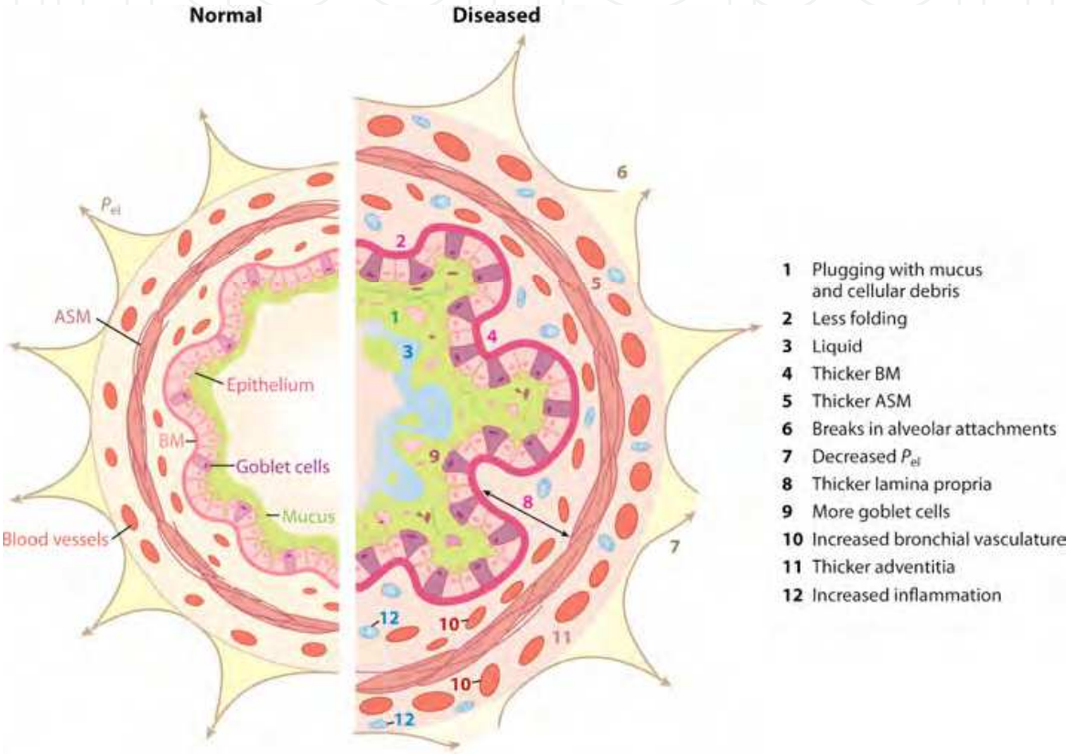

Fig. 3. A cartoon of an airway half normal (on the left) and half asthmatic (on the right) to illustrate the various features of airway remodeling in asthma. Airway responsiveness can be influenced by (1) an increase in volume of mucus and/or cellular debris in the airway lumen, (2) fewer but deeper folds in the mucosal membrane, (3) increased volume or altered surface tension of the liquid layer, (4) thickening and stiffening of the basement membrane (BM), (5) increased ASM mass embedded in an increased fibrous network, (6) decreased integrity and/or (7) decreased number of alveolar tethers to the adventitia contributing to decreased elastic recoil, (8) increased thickness of the lamina propria, (9) increased goblet cell number, (10) increased vascularity and/or vascular dilatation in the lamina propria and adventitia, (11) increased thickness of the adventitia, and (12) increased inflammatory cell infiltration throughout the airway wall. From Bossé *et al*. Annu Rev Physiol 2010; 72: 437-62.

4.1.1 Epithelium integrity

Researchers working with isolated airway segments *ex vivo* have long recognized that the concentration of spasmogens required for a given response is orders of magnitude greater (30 to 300-fold) when a chosen spasmogen is delivered on the mucosal side (within the lumen) compared to when it is delivered on the adventitial side (179). Mechanical removal of the epithelium in bronchial segments of dogs has also been shown to increase ACh sensitivity by 100 to 716-fold (159, 187). These observations suggested that the epithelium acts as a cellular barrier and establishes a large concentration gradient across the airway wall. A breach in the integrity of the epithelium would disrupt its barrier function and, consequently, increases airway responsiveness by facilitating the delivery of spasmogens to the ASM. In fact, association between epithelial permeability and airway responsiveness in humans has been previously reported (108); albeit this finding is not unanimous (197). Increased leakiness of the epithelium can be due to cellular damage and desquamation, which can be induced, for example, by eosinophil mobilization and activation into the airways. Disruption of cell-cell connectivity, by interrupting E-cadherin adhesion for example, can equally facilitate the delivery of spasmogens to the ASM by increasing the paracellular conductance (254). Many asthma stressors, such as allergens, ozone, particulate matters, smoke and occupational triggers have been shown to affect airway epithelial integrity, which may underlie their link with AHR. Alterations in epithelium integrity by these asthma stressors can be mediated directly, or indirectly by fostering inflammatory cell infiltration into the epithelium or by increasing the release of endogenous proteases (187).

In addition to act as a physical barrier, the airway epithelium can also play a direct role in controlling ASM contractility. The airway epithelium has been shown to produce several bronchoactive substances, such as lipooxygenase and cyclooxygenase products as well as nitric oxide. This subject was reviewed in the past (225) and will not be discussed in detail here. Briefly, the combined effect of these mediators seems to be bronchodilatory and may thus play a protective role against airway narrowing. Collectively, these observations suggest that any alteration in the lungs affecting the integrity of the epithelium or the release of epithelium-derived bronchoactive substances may influence airway responsiveness.

4.1.2 Vascular remodeling

The number and size of blood vessels, as well as the vascular area, are increased in asthmatic airways (reviewed in (55)). Signs of dilation, congestion, endothelium leakiness and abnormal wall thickness of the airway vessels have also been noted, which all alter by different manner and different extent the normal function of these vessels. The space occupied by the vessels conspicuously increases airway wall thickness, but may also do so by changing its turgidity. It may also modify the mechanical properties of the airway wall and affect the pattern of airway wall folding during airway narrowing. The detrimental effects of these structural changes are discussed in the following subsections (4.1.3 and 4.1.4).

Chronic vascular alterations also have the potential to exacerbate edematous changes occurring in the face of inflammation (discussed in section 4.2.2). It may also alter the clearance of spasmogens into the airways, which can greatly affect the degree of airway responsiveness (151). Taken together, these results suggest that changes in number, size, structure and integrity of the vascular bed interwinding the airway wall can have direct or indirect repercussions on airway responsiveness.

4.1.3 Increased amount of material inner the ASM layer

As aforementioned, all the layers of the airway wall were shown to be thicker in asthma. An increased amount of material inner the ASM layer amplifies the luminal airway narrowing for any given degree of ASM shortening. This is because of a geometrical effect. Airway resistance is inversely related to the luminal radius at the fourth power. So small decreases in radius can cause huge increases in airflow resistance. This effect is more pronounced when the thickening encroaches the lumen (inwardly-directed remodeling) prior constriction. This is because the airway lumen will be smaller to begin with. Thus, the same amount of ASM shortening (or the same decrease in luminal radius) would increase further the resistance to airflow. However, this geometric effect would also occur if the thickening does not encroach the lumen (outwardly-directed remodeling) prior constriction. This is because the luminal area decreases as the ASM shortens, but the area of the material inner the ASM does not; assuming that this material is uncompressible. With a greater fraction of the total area occupied by uncompressible material to begin with, a thicker airway wall would exaggerate the changes in the luminal area during ASM shortening. In other words, for a given change in total area (i.e., area inner the ASM layer), if more area is occupied by uncompressible material, more changes in the compressible area (i.e., lumen) would have to occur. Importantly, filling the airway lumen by inflammatory cells, mucus accumulation or by plasma extravasation would have the same detrimental consequence.

In vivo, even if the material inner the ASM would be somewhat compressible, an increased amount of material inner the ASM layer would still contribute to AHR, unless the compressibility of this material is severely affected in asthma. Computation models of the human bronchial tree confirmed that thicker airway wall inner the ASM increases airflow resistance caused by any given degree of ASM activation (126). This effect may not be small. Wagers and coworkers have developed a computational model based on morphometric and functional data derived from a mouse model of allergic airway inflammation to address this question. They predicted that thickening of the airway wall combined with an increased propensity for airway closure (discussed later in subsection 4.4.2) in the 'asthmatic' mice were sufficient to explain entirely AHR, without the need of increasing ASM shortening (237). Taken together, any lung defect that increase the volume of material inner the ASM layer leads to more luminal narrowing for any given degree of ASM shortening. This is a good example showing that AHR may be present despite normal ASM contractility.

4.1.4 Reduced ASM-load due to remodeling

The load impeding muscle shortening is just as important as the force-generating capacity of the ASM. A weaker load will allow the ASM to shorten more but also to shorter faster for the same reasons as a stronger ASM would shorten more and faster against a given load (discussed in subsection 2.1).

The loads impeding ASM shortening originate from different airway wall and lung elements. One of the most important load is the parenchymal recoil; i.e., the radial tethering force offered by the parenchymal attachments on the outer edge (adventitia) of the airway wall. In fact, this load seems to be crucial for the maintenance of intraparenchymal airway patency. In condition where the ASM would be fully relaxed, this load is totally counterbalanced by the circumferential tension of the airway wall; an inwardly-directed force equal to the force of recoil but acting in opposite direction. To overcome a greater recoil, more strain within the airway wall would be required to reach a circumferential tension equal but opposite to the recoil, which would mean more airway dilatation. Therefore, greater

parenchymal recoil leads to greater airway caliber. The extent of the recoil depends on lung volume, the elasticity of the parenchymal tissue and the strength of the connection between the airway wall and the parenchyma (the later is sometimes called the force of interdependence). Any lung defect affecting these parameters may influence the strength of the recoil at a given lung volume and, concomitantly, change airway caliber. The parenchymal recoil also offers an additional load during airway narrowing because the lung parenchyma is progressively distorted as the ASM shortens. This load can be calculated from the shear modulus (μ) of the lung parenchyma, which was estimated to be 0.7 time the transpulmonary pressure (124), and the changes in adventitial diameter during airway narrowing, which is geometrically related to the changes in ASM length during shortening (126). So in addition to keep the intraparenchymal airways patent prior constriction, the lung recoil imposes an after-load that further limits airway narrowing once the ASM begins to shorten. Lung defects previously observed in asthma, such as alveolar breaks (149) and adventitial thickening (190), were shown to decouple the airways from the parenchyma and affect the extent of both the pre- and the after-load offered by the recoil. These lung defects may contribute to AHR.

The ECM within and surrounding the ASM bundle also affects the after-load impeding muscle shortening. In conditions where the muscle is fully relaxed, the circumferential tension (which is carrying the load of the recoil) would be born from the passive elements of the airway wall, including the resting tension of the ASM. However, during narrowing induced by ASM shortening, the load carried by the parallel elastic elements is progressively transferred to the ASM. This transfer proceeds until these parallel elastic elements are no longer in tension but in compression. At that time, they become a direct load impeding muscle shortening. So whether these parallel elastic elements are in tension and progressively transfer the load to the ASM during airway narrowing or whether they pass the transition from being in tension to become in compression, they still limit ASM shortening by increasing the after-load. If this material is stiffer for example, it would be carrying more load for less strain (i.e., for a given lung recoil pressure there will be less airway dilatation). In those conditions, while the ASM begins to shorten upon activation, a greater load would be transferred to the ASM for a given amount of airway narrowing. It will also be harder to compress this material if airway narrowing proceeds until the parallel elastic elements are no longer in tension but in compression. In addition, when a cell or a bundle of ASM shortens, its center tempts to bulge. Anything that would prevent the modification of cell shape required for shortening may limit airway narrowing. This load has been called radial constraint (190). Together, this suggests that the quantity and the mechanical properties of the ECM, but also any other wall constituents, within and surrounding the ASM cells or bundles may affect the degree of airway narrowing. In support of this contention, pre-treatment of airway wall strips with collagenase was shown to increase ASM shortening in response to a given contractile stimulus (29). This result suggested that degradation of the collagenous matrix in the airway wall reduces the load, allowing the ASM to shorten further in response to a given level of activation.

Epithelial buckling pressure and airway wall corrugation that occur during airway narrowing are also believed to provide an after-load hampering ASM shortening. The number of mucosal folds developing during narrowing seems to be of major importance. The number of folds, in turn, may be determined by the flexibility, stiffness and thickness of the airway wall, by the stiffness and thickness ratios of the different layers composing the airway wall, or by structural features such as longitudinal elastic bundles (37) or blood and lymphatic vessels (239), which could dictate the location of the folds. More studies are

warranted to establish a consensus concerning the factors influencing the pattern of mucosal folding and how it affects ASM-load. Current evidences suggested that the number of folds is not different between asthmatic and non-asthmatic airways (37).
The load impeding ASM shortening may either decrease or increase due to airway remodeling. Thickening and/or fibrosis of the airway wall, for instance, may protect against airway narrowing. Accordingly, airway reactivity was shown to correlate negatively with airway wall thickness in humans with asthma (155, 175). Similar observations were made in a mouse model of asthma (2). Therefore, even if thickening due to fibrosis can theoretically increase airway narrowing in response to spasmogens because of geometrical effects, airway wall stiffening due to thicker or more fibrotic wall may well protect against AHR. Clearly, it is not just the geometric effects of wall thickening that influence airway narrowing (189). The mechanical properties of the material that causes the thickening are also important since ASM has to deform this material to narrow the lumen. The composition of the ECM may also affect the ASM phenotype (discussed in subsection 4.1.6).
Taken together, we have seen that the caliber of the airways embedded in the parenchyma is determined by the dilating force of the lung elastic recoil and the stiffness of the airway wall. Anything affecting the parenchymal recoil, the airway-parenchymal interdependence, the parallel elastic elements, the radial constraint and the pattern of mucosal folding may reduce (or augment) the load and, consequently, causes more (or less) ASM shortening for any given degree of ASM activation.

4.1.5 Length adaptation

A decrease in airway caliber caused by any lung defect attenuating the pre-load would cause a reduction in ASM length. Because the force-generating capacity of the ASM is proportional to its length, decreasing ASM length simultaneously decreases its force-generating capacity. However, the ASM is endowed with an intrinsic ability to adapt to length changes (reviewed in (27)). This phenomenon was called length adaptation and is defined as the recovery of ASM force-generating capacity that was loss following length perturbations. Adaptation of ASM to a shorter length is particularly relevant to the understanding of AHR. This is because a muscle adapted to shorten length is able to generate more force at any given length during shortening comparatively to a muscle adapted to a longer length. In contradistinction, adaptation to longer length would have a protective effect in terms of airway responsiveness. This is a good example to show how a normal ASM behavior (i.e., length adaptation), may contribute to AHR by increasing a contractile property of the ASM (herein force).
Length adaptation is likely to occur in different situations. Emphysema, for example, decreases airway caliber by reducing lung recoil, which will allow the ASM to adapt to shorter length. Other factors reducing lung volume such as recumbent posture (247), high spinal cord injury (201) and obesity could have a similar effect. However, a recent review on obesity and AHR (206) rather concludes that obesity has little direct effect on airway caliber despite reducing functional residual capacity (FRC) and expiratory reserve volume. Based on the authors, the expiratory flow limitation observed in individuals with low lung volume (like the obese) is due to an increased propensity for airway closure (discussed in subsection 4.4.2). That may also apply to other conditions affecting lung volume.
Another contractile property that is affected by length adaptation is the ASM-passive stiffness. It was shown that the passive stiffness initially declines following a length reduction but slowly re-develops over time (28). However, since the contribution of ASM-

passive stiffness to the overall stiffness of the airway wall is neglectable, it is insignificant to the understanding of AHR in asthma.

4.1.6 Alterations in the composition to the ECM

It was suggested that the phenotype of ASM cells can be switched around from a contractile phenotype to a synthetic/proliferative phenotype (95). ECM components are thought to play an important role in determining ASM phenotypes. Laminin, for example, was shown to be required for the transformation of ASM cells into a more contractile phenotype (233). The existing interplay between ECM and ASM phenotypes and functions has been the subject of other reviews (232). It is known that the composition of the ECM is altered in asthma due to either a change in the rate of ECM component synthesis or a change in the rate of their degradation, the later being controlled by a balance between proteases and their inhibitors. Taken together, this suggests that a fine balance of ECM components is required to keep the ASM phenotype in check. Any alteration in this balance may affect ASM contractility and airway responsiveness.

4.1.7 Enlargement of ASM mass

We came to realize that the mass of ASM is increased in asthmatic airways almost a century ago (106). This structural change was one of the first hypothesis advanced to explain AHR in asthma and has not been refuted yet. Although a recent study suggested that the increased ASM mass is related predominantly to cellular hyperplasia rather than hypertrophy (199), the exact origin of ASM enlargement is still a matter of debate. In addition to the possibilities of cellular hyperplasia and hypertrophy, alternative hypotheses have been proposed, such as myositis (i.e., inflammation of the ASM) and increased ECM deposition. The origin of hyperplasia is also unclear but could stem from increased proliferation, decreased rate of apoptosis and/or migration of ASM progenitors into the ASM bundle with their subsequent differentiation into ASM cells (reviewed in (25)). The role of proliferation in ASM hyperplasia has gained credibility recently when Hassan and coworkers (100) provided data suggestive of an increased rate of ASM cell proliferation in humans with severe asthma. Irrespective of the mechanism of ASM thickening, the increased mass could lead to a greater total force generation for any given level of ASM activation; assuming that the ASM from asthmatics produce the same stress (force per cross-sectional area) as non-asthmatic ASM. *In vivo*, that would be translated into increased airway narrowing for any given dose of inhaled spasmogen and, therefore, AHR.

To reiterate, asthmatic remodeling is characterized by relatively permanent changes in the architecture of the airways, the lung parenchyma and their interconnection. Many of these changes can affect, positively or negatively, the degree of airway responsiveness. The integrity of the epithelium and vascular remodeling may influence the delivery and the clearance of spasmogens toward and away from the ASM, respectively. Increased amount of material inner the ASM layer can increase airway narrowing by a geometrical effect. Decremented load impeding ASM shortening can cause more ASM shortening for any given degree of ASM activation. Remodeling can also promote ASM hypercontractility by fostering normal ASM behaviors such as length adaptation or phenotype switching. Finally, factors fostering the growing of the ASM mass encircling the airways may increase ASM-force per unit of airway length. Those are all different ways by which a normal ASM may account for AHR in a remodeled environment.

4.2 Airway inflammation

Airway inflammation is a hallmark of asthma. The use of the term 'airway inflammation' is vague though. Asthma can be characterized by different types of inflammation, which may be useful to distinguish for diagnosis purposes and to offer patients the optimal therapeutic strategy (248). There is clearly a link between airway inflammation and AHR. Interventions altering airway inflammation are effective in changing the degree of airway responsiveness. For example, AHR can be increased in asthmatics following the induction of acute inflammation by inhalation of allergens (47, 143) or IL-5 (218). On the other hand, reducing inflammation by the use of glucocorticoids attenuates AHR (reviewed in (30)).

Airway inflammation can affect airway responsiveness by many means. It is sometime hard to dissociate the effect of inflammatory changes from remodeling changes, since both can affect features of the airway wall, such as its thickness, that participate to AHR. It is also hard to dissociate because of the interplay that exists between inflammation and remodeling. Many believe that inflammation is the cause of remodeling, as the damage caused by inflammation may lead to impaired repair, scaring and loss of function. For the sake of this chapter, we considered inflammation as the transient and potentially reversible changes occurring in the lungs of asthmatics following exposure to the triggering agent. We also consider the direct influence of asthma triggers and inflammation-derived molecules on ASM contractile properties.

4.2.1 Mucus accumulation

Any encroachment of the airway lumen, whether it is provoked by narrowing or by filling the lumen, causes airway obstruction, increases airway resistance and contributes to AHR. The mucus layer is required for normal lung homeostasis. It is recognized as a physical and immunological barrier protecting the host against inhaled pathogens, particulates and pollutants. However, its hyper-secretion can encroach the lumen and causes airway obstruction. Goblet cell hyperplasia (1), as well as enlargement and hyperplasia of the submucosal glands (16), can contribute to mucus hyper-secretion. These are remodeling changes but their contribution to lung dysfunction is only manifested upon exposure to airway stresses. In the extreme cases, mucus can also cause complete occlusion by forming mucus plugs, which contain, in addition to mucus, proteinaceous exudate, inflammatory cells and isolated epithelial cells and creola bodies (123). Complete occlusion of some airways severely compromises airflow into the lungs, and may thus be an important player in the manifestation of AHR. It also has severe repercussions on ventilation.

The accumulation of mucus can also originate from improper clearance. Mucociliary clearance was shown to be slower in asthmatics (108). The cephalad transport of mucus into the lungs is motored by a synchronized rotational beating of the epithelial cils and by cough when necessary. It also relies on the integrity of a low-viscosity solution lying atop the epithelial cells called the periciliary liquid layer (PCL), which prevents the shear friction between the epithelial surface glycocalyx and the mucus layer (120). Any alteration in mucus clearance, such as cil diskinesia, changes in mucus consistency, overly thin PCL and problems associated with cough reflex can all lead to mucus accumulation and airway obstruction. In addition, stagnant mucus can increase the risk of infection (120).

Mucus accumulation also increases the surface tension. It was estimated that replacing the normal fluid lining the epithelium of peripheral airways by mucus would increase surface tension by 5 folds (141). Surface tension is sometime overlook but can play an important role

in determining the degree of airway responsiveness. This was well demonstrated when we came to realize that the pressure required to achieve a given lung volume is significantly higher when the lung is inflated with a gas compare to when it is filled with a liquid. In addition to its great impact on airway resistance, surface tension also increases the propensity for airway closure. In fact, a 5-fold increase in surface tension was predicted to be sufficient to cause airway instability and collapse (141). The influence of airway closure in airway responsiveness is discussed further in subsection 4.4.2. The role of surfactant in controlling surface tension is thus of major importance. Any alteration in surfactant caused by allergen exposure (51) or plasma exudation (238) is likely to affect surface tension and, concomitantly, lung functions (257). Finally, combined with ASM constriction, the baseline airway obstruction induced by mucus accumulation can synergistically increase airway responsiveness for geometric reasons mentioned in section 4.1.3.

4.2.2 Edema

Edema is a characteristic feature of inflammation. It is due to an increased permeability of the vessels. This increased leakiness of the vascular endothelium swells the tissue by fostering extravasation of inflammatory cells, as well as plasmatic fluid and proteins, into the interstitial tissue. It may also encroach the airway lumen. Plasma exudation onto the epithelial lining is increased in asthma and the extent of it is associated with the level of airway responsiveness (235). Many inflammatory molecules that are overexpressed in asthma were shown to increase vascular permeability, such as substance P, histamine and many others. Newly formed blood vessels are also more permeable than the existing vessels (10). Angiogenic remodeling seen in asthma may thus render the airways more prone to tumefaction.

Edema can alter the degree of airway responsiveness by many means. Plasma exudation induced experimentally by infusion of saline in dogs was shown to increase airway wall thickness and to decrease the caliber of the airway lumen over the level achieved simply by increasing the vascular pressure (35). This model of airway edema suggested that both the airway wall thickness and the size of the lumen are affected at baseline by airway turgidity. These changes in lumen and airway wall geometry can severely increase the degree of airway responsiveness as discussed in section 4.1.3. In fact, using this dog model of airway engorgement by saline infusion, it was shown that airway responsiveness to histamine increases for the same level of ASM shortening (34). Leakage of plasma protein in the epithelial lining fluid can also be detrimental for airway narrowing and closure. Fibrin, for example, inactivates the surfactant and can increase airway responsiveness by increasing the surface tension (238).

4.2.3 Inflammatory cells and molecules

In addition to mucus accumulation and edema, which increase airway responsiveness mainly by changing the luminal and airway wall geometry, other inflammatory changes may increase directly ASM contractility.

At the cellular level, mast cells have been linked to increased ASM contractility. The number of mast cells has been shown to be elevated in ASM tissues of asthmatics compared to non-asthmatic subjects with or without other inflammatory disorders (31). The participation of mast cells in AHR has been suggested based on correlations observed between the percentage of mast cells recovered in the bronchoalveolar lavage fluid (BALF) (64) or

interspersed in the ASM tissue (113) and the degree of airway responsiveness. Interestingly, the later correlation was even stronger when the mast cells with a fibroblastoid phenotype, which spontaneously produce more histamine, were counted (113). It was also demonstrated using immunohistological sections of human airways that ASM cells that localized in vicinity of mast cells express higher levels of α-smooth muscle actin (α-SMA), suggesting a paracrine influence of mast cells that increases ASM contractility (255). Taken together, these results suggest that the release of mast cell-derived mediators or direct mast cell-ASM contact could contribute to AHR by making the ASM stronger.

An earlier study also suggested that a short period of mast cell activation alone is sufficient to induce AHR in mice. In that study, it was shown that acute activation of mast cells by an anti-IgE antibody 20 min prior to methacholine (MCh) challenge increases airway responsiveness in wild-type naïve (i.e., non-sensitized and challenged) animals but not in mast cell-deficient naïve animals (146). Apart from histamine, mast cells can release a plethora of inflammatory mediators upon activation. As it is the case for histamine, some of these mediators increase ASM-tone directly by triggering ASM contraction, such as prostaglandin D_2 and leukotrienes. It would be a hard task to distinguish the effect of inflammation from the effect of increased ASM-tone on airway responsiveness. This is because a lot of molecules that are part of the asthmatic inflammatory processes are spasmogens. The increase ASM-tone caused by inflammation-derived spasmogens will be discussed separately in a following subsection (4.3). In the present subsection, the focus is on the inflammatory mediators that are not spasmogenic but have been shown to increase ASM contractility.

At the molecular level, several asthma triggers have been shown to increase ASM contractility *ex vivo*. For example, ASM contractility has been shown to be enhanced following prolong (at least 16 h) incubation with atopic serum (15, 19, 78, 89, 91, 161, 211, 245, 246), IgE immune complex (80, 89, 250) and exogenous asthma triggers such as the house dust mite allergen *Der p 1* (82), the bacterial endotoxin lipopolysaccharide (LPS) (8, 166, 216, 220) and the rhinovirus (serotype 16) (78, 81) or the virus mimetic toll-like receptor (TLR)3 ligand polyinosinic polycytidylic acid (poly-IC) (8). Most of these studies assessed ASM contractility by measuring its force-generating capacity. However, some studies also reported that some of these inflammatory insults change the velocity and amount of shortening of the ASM in response to a spasmogen (161), as well as its ability to relax in response to bronchodilators (8, 15, 78, 81, 89, 216). The half-time of relaxation after short EFS-induced tetani has also been shown to increase (double) in ASM strips derived from dogs sensitized to ragweed (109).

The effect of asthma triggers on *ex vivo* ASM contractility can be indirect; i.e., due to an autocrine loop of mediators that are produced by the ASM in response to asthma triggers (79, 80, 91, 166). *In vivo*, the paracrine influence of other cells that are responsive to asthma triggers can also impact ASM contractility. Ultimately, all the inflammatory mediators overexpressed in asthma, whether they originate from other cells or from the ASM itself, can contribute individually or in combination to ASM hypercontractility and AHR. Supports for this contention are accumulating. Cytokines such as interleukin (IL)-1β (11, 92, 200, 250), tumor necrosis factor (TNF)α (4, 7, 167, 168, 172, 191, 193, 200, 227), the combination of TNFα and IL-1β (90, 92), IL-13 (41, 62, 80, 228), IL-5 (93, 250), IL-10 (79), granulocyte-macrophage colony-stimulating factor (GM-CSF) (94), interferon (IFN)γ (5), leukemia inhibitory factor (LIF) (63, 119) and transforming growth factor (TGF)β (75, 255), as well as

the protease β-tryptase (212, 255) were shown to increase ASM contractility. In turn, the effect of these mediators is not always direct. For example, the potentiating effect of IL-1β on bradykinin-induced contraction is due to a greater release of thromboxane (Tx)A_2 in response to bradykinin (166). As for the asthma triggers, most of these studies assessed ASM contractility by measuring its force-generating capacity. However, inflammatory mediators found in asthmatic airways can also impair ASM relaxation. For example TNFα (90, 92), IL-1β (90, 92), IL-13 (127), IL-10 (79), IL-5 (79) and lysophosphatidic acid (LPA) (230) have all been shown to attenuate ASM relaxation elicited by β_2-adrenoceptor agonists. In addition, TNFα (9, 92) and IL-1β (92) were shown to attenuate the relaxant effect of prostaglandin (PG)E_2.

The molecular mechanisms governing the transformation of ASM into a hypercontractile phenotype may differ from one inflammatory mediator to another. In the case of TGFβ, increased expression of the contractile protein α-SMA and actin filamentogenesis have been proposed (75, 76). On the other hand, many inflammatory mediators increase ASM contractility through shared mechanisms related to alterations in calcium handling; either by Ca^{2+} sensitization via the Rho-ROCK pathway or by increasing the intracellular mobilization of Ca^{2+} via the CD38/cADPR/RyR pathway (both briefly described below and illustrated in Figure 4).

Ca^{2+} sensitization is a phenomenon that allows the ASM to produce more force in response to a given mobilization of intracellular calcium concentration ($[Ca^{2+}]_i$). Ca^{2+} sensitization seems to rely mainly on a signaling pathway running in parallel to canonical Ca^{2+} signaling pathways (inositol 3-phosphate receptor (IP_3R)-dependent and entry from the extracellular compartment). The pathway is referred to as the Rho-Rho-associated, coiled coil-containing kinase (ROCK) pathway and is activated by certain G protein-coupled receptors (GPCRs) following ligation with their cognate spasmogen (see Figure 4). Rho is a small G (GTPase) protein activated by the exchange of GDP for GTP. The identity of the guanine exchange factor (GEF) that is activated by the GPCR and involved in Rho activation is unclear, and is probably receptor specific. However, one of the downstream signals mediated by Rho that leads to Ca^{2+} sensitization is well characterized. GTP-bound (active) Rho initially activates ROCK, which then inhibits myosin light chain (MLC) phosphatase (MLCP) both directly, via phosphorylation of the myosin phosphatase-targeting subunit 1 (MYPT1) of MLCP, and indirectly, via CPI-17 (17-kDa PKC-potentiated inhibitory protein of PP1; PP1 stands for protein phosphatase 1, which is the catalytic subunit of the heterotrimeric MLCP). Since the level of MLC phosphorylation depends on a balance between its phosphorylation by MLCK and its dephosphorylation by MLCP, direct or indirect inhibition of MLCP by ROCK causes more MLC phosphorylation for the same degree of MLCK activation. Therefore, when the Rho-ROCK pathway is activated, the same degree of MLCK activation induced by a given sarcoplasmic Ca^{2+} mobilization leads to more MLC phosphorylation and sequentially more activated cross-bridges and more force.

AHR in animal models of airway inflammation has been attributed to an increased ASM-sensitivity to Ca^{2+} (40, 43). Ca^{2+} sensitization is more typically triggered by inflammation-derived spasmogens (discussed in section 4.3.1), which bind to GPCRs capable of activating the Rho-ROCK pathway. However, non-spasmogenic inflammatory mediators such as TNFα have also been shown to potentiate ASM-force by fostering Ca^{2+} sensitization (107, 172, 191). In addition, the extent of Ca^{2+} sensitization can be regulated by mediators affecting the levels of expression or activation of key regulatory proteins involved in the Rho-ROCK pathway. For example, IL-13 (41) increases the level of RhoA expression in ASM, which consequently increases the proficiency of Rho-ROCK signaling pathway to induce Ca^{2+}

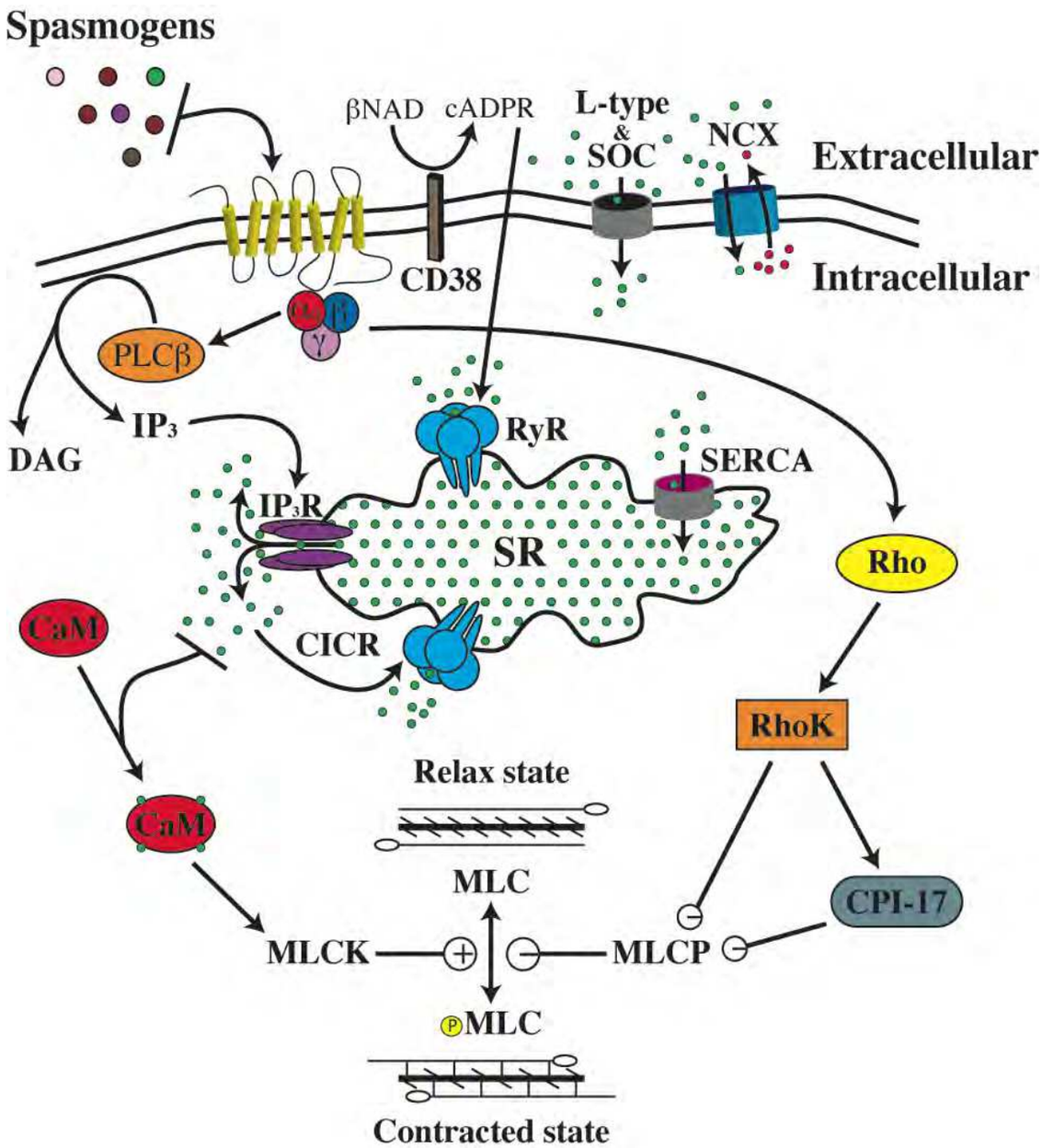

Fig. 4. Pathways transducing the extracellular signal of the spasmogens from their cognate cell-surface receptor to the contractile apparatus. See text for descriptions and abbreviations. The green and red dots represent Ca^{2+} and Na^{+}, respectively. The reverse mode of the Na^{+}/Ca^{2+} exchanger (NCX) is shown. Reproduced with permission from: Bossé and Paré. Airway smooth muscle responsiveness: The origin of airway hyperresponsiveness in asthma? Current Respiratory Medicine Reviews. 7(4): 289-301, 2011.

sensitization. On the other hand, mediators capable of reducing the expression/activity of intermediate proteins involved in this pathway may have therapeutic potential for diminishing Ca^{2+} sensitization and AHR. One example is the effect of glucocorticoids in reducing the expression of RhoA, which concomitantly attenuates antigen-induced ASM hyperresponsiveness in a rat model of allergic airway inflammation (40).

In addition to canonical Ca^{2+} signaling pathways, at least one other Ca^{2+} signaling pathway contributes to the release of Ca^{2+} into the sarcoplasm (see Figure 4). This pathway is referred to as the CD38/cyclic adenosine-5'-diphosphate (ADP) ribose (cADPR) signaling pathway and has received significant attention recently in regard to its potential role in AHR. CD38 is a type II transmembrane protein of 45-kDa with dual ecto-enzymatic activity. It possesses an ADP-ribosyl cyclase activity, converting β-NAD into cADPR and a cADPR hydrolase activity, converting the cADPR into ADPR. The product of the ADP-ribosyl cyclase activity, cADPR, sensitizes the ryanodine receptor (RyR) for Ca^{2+} release and, more specifically, potentiates Ca^{2+}-induced Ca^{2+} release (CICR) (70). Whether cADPR binds directly to RyR or requires other intermediates to open the RyR channel is still a matter of debate (54). What is sure is that cADPR increases the open probability of the RyR, liberating more Ca^{2+} from the sarcoplasmic reticulum (SR) (196). As is the case for IP_3R-dependent Ca^{2+} release, the CD38 pathway is also activated by binding of specific spasmogens to their cognate receptors (252). Thus, this pathway acts in parallel with the canonical Ca^{2+} signaling pathways to amplify the mobilization of Ca^{2+} into the sarcoplasm following spasmogenic stimulation.

The amplitude of Ca^{2+} release by the CD38/cADPR/RyR pathway, as well as its contribution in ASM-force generation, is influenced by CD38 expression. CD38 expression and/or activity were shown to be upregulated by many of the pro-inflammatory cytokines present in asthmatic airways, such as IFNβ (229), IFNγ (53), TNFα (53, 229), IL-1β (53) and IL-13 (52). Therefore, in the presence of inflammation, the proficiency of this signaling pathway to liberate the Ca^{2+} from the internal stores may be enhanced. Consequently, higher force generation would be attained for a given contractile stimulus since more Ca^{2+} would be released. The influence of inflammatory mediators on the CD38/cADPR/RyR pathway may thus play a role in AHR seen in asthma.

Taken together, these results suggested that the contractile properties of ASM are not fixed but rather plastic; i.e., they can change rapidly in the face of external (inflammatory) cues. So in an inflammatory lung disorder such as asthma, the ASM may be stronger or faster, for example, because of the 'bad' environment in which it is embedded. This increased contractility may only be present *in vivo* when the muscle is exposed to inflammation-derived mediators, but might be lost once the muscle is removed from the airways and washed repetitively before being studied *ex vivo.* These results may offer an explanation for the failure of *ex vivo* studies to show an increased strength of ASM from asthmatics (reviewed in (153)). The ability of ASM to rapidly change its contractile capacity in response to inflammatory molecules can also contribute to changes in airway responsiveness observed in response to interventions altering airway inflammation (30, 47, 143, 218). Those are all different ways by which a normal ASM may account for AHR in an inflamed environment. Another way by which inflammation can increase airway responsiveness is by producing spasmogens, which increases ASM-tone.

4.3 Increased ASM-tone

The responsiveness of asthmatics to bronchodilators, together with more direct evidence (165), indicate that ASM-tone is increased in asthmatic airways. The origin of this

augmented ASM-tone is not clear and likely varies between asthmatic individuals. Nonetheless, asthmatic lungs are characterized by the overexpression of inflammation-derived spasmogens. These spasmogens trigger sustained ASM contraction and, acting individually or collectively, are a likely cause of increased ASM-tone in asthma. In the present subsection, we discuss the mechanisms by which inflammation-derived spasmogens, together with the attendant increase in ASM-tone, can contribute to AHR.

The rationale is that the amount of airway narrowing in response to a given concentration of a chosen spasmogen (e.g., MCh) is due to the combined effect of this extrinsically delivered spasmogen plus the baseline tone, which is caused by the endogenous spasmogens already present. In other words, it is the cooperative effect of all the spasmogens present at the time of the challenge that determines the total level of ASM activation and, ultimately, the amount of airway narrowing achieved. In addition, one of the causes of AHR can be the contractile synergistic interactions that are often observed when different spasmogens are used in combination. In this regard, a sizable literature exists documenting contractile synergisms between different spasmogens (Table 1). The mechanisms involved in these synergistic interactions have been debated in the last few decades and it is now clear that all the mechanisms that may be involved are not mutually exclusive. The nature of these interplays can give us important clues regarding the role of baseline airway tone in determining the degree of airway responsiveness.

4.3.1 Interactive synergisms between spasmogens

Initial studies in that area begin in the late 1970s and have started by looking at the combined effect of spasmogens on different measures of lung function in animals (45, 46, 57, 99, 102, 112, 131, 152, 180, 181, 192, 208, 209, 224, 244), as well as in humans (49, 68, 97, 101, 110, 156, 158, 194, 202, 240). Apart from an interactive synergism at the level of ASM contraction, many other factors can account for these *in vivo* interactive synergisms. In fact, although these synergistic interactions can be extremely relevant to the understanding of AHR, the mechanisms involved are sometime impossible to delineate.

First, the interactions between spasmogens were assessed *in vivo* by measuring airway resistance (or conductance) and/or flow parameters (flow rate at different lung volumes, forced expiratory volume in 1 sec (FEV1), etc...). All of these measurements are geometry-dependent. As mentioned earlier, airway resistance is inversely proportional to the luminal radius at the 4^{th} power. The potentiation of the effect of one spasmogen by another spasmogen (or an increased baseline tone) might be related to a geometrical effect more than a true synergistic interaction of spasmogens on ASM-force. In other words, an additive effect at the level of muscle activation that may result in an additive effect in terms of ASM-force generation and shortening can be perceived as a synergistic effect when looking at airway resistance.

To circumvent these limitations, others have used *in situ* preparations allowing the assessment of tracheal smooth muscle-isometric force (12, 33, 131) or have measured airway caliber using tantalum bronchography (17). Some of these studies confirmed the synergistic effect of spasmogens on airway narrowing and ASM-force generation. However, these results are also confounded by the acute effect of some spasmogens on vessels and nerves, or by the effect of some spasmogens on airway inflammation and the epithelium. In regard to the vessels, many airway spasmogens also have vasoactive properties. Both vasoconstrictors and vasodilators have significant impact on the degree of airway

Mediators	***in vivo***	***ex vivo***	***in vitro***
ET-1	CCh (180) in sheep; HIST (112) in pigs		
Muscarinic agonists (ACh, MCh or CCh)	HIST (158) in humans; HIST (131) and NE (33) in dogs	HIST (71), 5-HT (71) and NE (87, 185) in canine tracheal SM strips	
HIST	MCh (158) and HIST (97) in humans MCh (131), ACh (33) and NE (12, 33, 131) in dogs	MCh (71), ACh (87), 5HT (87) and NE (12, 87) in canine tracheal SM strips; CCh (135) in bovine tracheal strips	
AII	MCh (156) in humans; MCh (244) in guinea pigs	MCh (156) in human bronchi; ET-1 (32, 173, 174, 195) in bovine bronchi; CCh (203, 204), K^* (204) and NaF (204) in rat bronchial rings	
5-HT	NE (12, 33), HIST (57), ACh (57) in dogs	MCh (71), ACh (87) and NE (12, 87) in canine tracheal SM strips; CCh (135) in bovine tracheal strips	
NE (in the presence of β-adrenoceptor brockade)	HIST (131) in dogs; HIST (208) and bombesin (208) in guinea pigs		
ATP		MCh (184) in guinea pig tracheal strips	
Adenosine	HIST (224) in guinea pigs		
Dopamine	HIST (208) and bombesin (208) in guinea pigs		
TXA_2 (or its pharmacological analog U46619)	MCh (110) in humans		
PGD_2	HIST (68, 97) and MCh (68) in humans		
PGE_2		CCh (38) in bovine tracheal strips	
$PGF_{2\alpha}$	HIST (101, 240) in humans	CCh (38) in bovine tracheal	
8-isoprostanes	MCh (102) in mice	K^+ (38), CCh (38, 135) and histamine (38) in bovine tracheal SM	
PAF	MCh (49, 202) in humans; LTD_4 (192) in rhesus monkeys; ACh (46) in dogs; HIST (45) in sheep; HIST (152) and bombesin (209) in guinea pigs		

Mediators	*in vivo*	*ex vivo*	*in vitro*
LTB_4	ACh (181) in dogs		
Cys-LTs	HIST (194) and PGD_2 (194) in humans; PAF (192) in rhesus monkeys	K^+ (215) and CCh (215) in porcine tracheal strips; ET-1 (195) in bovine bronchial rings; ACh (48) and HIST (48) in guinea pig tracheal rings	
S1P		MCh (122) in guinea pig tracheal strips; K^+ in bronchial rings from normal but not antigen-challenged mice (42)	
LPA		MCh (230), 5-HT (230), SP (230) in rabbit tracheal rings; MCh (230) in cat tracheal rings; ACh (98) in guinea pig tracheal rings	ATP (205) and K^+ (205) in bovine tracheal SM cells (205)
PA		MCh (230) in rabbit tracheal rings	

Abbreviations in the table: AII, angiotensin II; ACh, acetylcholine; ATP, adenosine triphosphate; BK, bradykinin; CCh, carbachol; Cys-LTs, cysteinyl-leukotrienes; ET-1, endothelin-1; HIST, histamine; 5-HT, 5-hydroxytryptamine (also called serotonine); K^+, potassium; LPA, lysophosphatidic acid; LTB_4, leukotriene B_4; MCh, methacholine; NaF, sodium fluoride; NE, norepinephrine; PA, phosphatidic acid; PAF, platelet activating factor; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SM, smooth muscle; SP, substance P; S1P, sphingosine-1-phosphate; TXA_2, thromboxane A_2.

Table 1. Interactions between spasmogens increasing ASM contractility

responsiveness (151). The potentiating effect of histamine on the responsiveness to muscarinic agonists (33, 131), for example, can likely be due to its vasodilating effect, especially when the drugs are administered intravenously or via the tracheal vasculature. This is because vasodilatation facilitates the delivery of the bronchoactive substance (ACh) to the ASM. Histamine also increases airway epithelium permeability by altering E-cadherin-based adhesions (262). The effect of a second inhaled spasmogen may thus be potentiated by histamine pre-treatment because the second spasmogen will have easier access to the ASM.

In regard to the nerves, many studies have shown synergistic interactions between spasmogens and electrical stimulation of the vagal nerves (17, 33, 57, 88, 114, 137, 217, 258). Similarly, a decrease in responsiveness to spasmogens (other than ACh (88)) was observed following vagotomy (59, 137, 157, 258), cooling of the vagal nerves (58, 88), or treatments with either atropine (59, 219), tetrodotoxin (219) or hexamethonium (59). These results suggest that the basal cholinergic tone synergistically interacts with the exogenously delivered spasmogen. These studies are not included in Table 1 because it is not clear whether the potentiating effect is due to a true synergistic effect at the level of ASM-force generation or due to the ability of the spasmogens to either trigger a vagal reflex (36, 58, 74, 157) or to increase cholinergic neurotransmission and/or synaptic transmission at the

ganglia (217). These latter phenomena would lead to more ACh release and concomitantly higher ASM activation for the same neural input. On the other hand, the bronchoconstricting effect of spasmogens is known to decrease the vagal tone (13, 210). This is because of their indirect effect on the slowly adapting stretch receptors (SARs), which, upon an enhanced discharge, relax the ASM by reducing parasympathetic tone to the airways. However, other spasmogens, such as bradykinin, would synergize with the effect of these mechanoreceptors via C-fibers activation to initiate the bronconstricting reflex (150). Together, the influence of the nervous system is intricate and certainly a major confounding factor *in vivo* when one tries to elucidate the synergistic interaction between spasmogens.

Finally, thickening of the airway wall by edema and inflammatory infiltrates, encroachment of the airway lumen by mucus hypersecretion and cellular debris, and reduction in the forces of interdependence between the airway wall and the lung parenchyma by the accumulation of inflammatory exudates in the adventitial layer are all means by which airway inflammation can increase airway responsiveness without affecting ASM-force (reviewed in (26)). Therefore, the hyperresponsiveness observed in response to the second spasmogen, when the first spasmogen is administered hours before the second spasmogen (180), may be due to the inflammation provoked by the initial spasmogen.

To avoid these confounding factors and to investigate whether the synergistic action of two spasmogens on airway responsiveness relies on mechanisms operating at the level of ASM, the only solution is to study the ASM tissue in isolation. Many investigators have focused on this strategy. Most studies were performed using freshly dissected ASM strips or bronchial rings from which isometric force was measured *ex vivo* (12, 32, 38, 48, 71, 87, 98, 122, 135, 156, 173, 174, 184, 185, 195, 203, 204, 215, 230). *In vitro* preparations (i.e., cell culture) have also been used on occasions (205). These reported synergisms are also enumerated inTable 1.

The molecular mechanisms involved in contractile synergisms observed between different pairs of spasmogens have been extensively studied. The synergisms involve the co-activation of ASM by two spasmogens acting on two distinct GPCRs. The activation of two distinct GPCRs can lead to intracellular signaling synergisms that ultimately potentiate ASM-force. The more commonly discussed mechanism is Ca^{2+} sensitization, defined as an increased ASM-force generation in response to a given amount of intracellular Ca^{2+} mobilization (as discussed in subsection 4.2.3). Many spasmogenic mediators, such as leukotriene C_4 (215), isoprostanes (135), ATP (184), endothelin-1 (260), LPA (205), TxA_2 (167) and S1P (122), have been shown to increase the responsiveness of the contractile apparatus to Ca^{2+}.

However, these synergisms in terms of force generation can also be related to an additive effect in terms of ASM activation, especially when the concentrations of spasmogens used are low. This is because the dose-response curve to any spasmogen is sigmoidal, regardless of whether the response is measured in terms of ASM-force or shortening. That means that initially, a threshold concentration is needed before any response can be measured, but then there is a progressive increase toward a steeper, relatively linear slope followed by a progressive decrease in slope to reach a plateau. When two different spasmogens are used in combination, their additive effect on ASM-activation could be erroneously interpreted as being synergistic because sub-threshold concentrations of two spasmogens could have a measurable effect on ASM-force or shortening. Similarly, if one uses a concentration just above the response threshold, the subsequent addition of a different or the same spasmogen will have more effect simply because it is now acting on the steeper part of the dose-response curve. In fact, many of the previously documented examples of 'synergism'

performed either *in vivo* (110, 112, 131, 158, 244) or *ex vivo* (38, 71, 87, 135) were involving low concentrations of spasmogens, and were unable to demonstrate a synergism at higher concentrations for at least some of the reported spasmogenic interactions (71, 87, 131). So the level of basal tone, even if low, can seemingly synergize with the extrinsically delivered spamogen to cause AHR simply by an additive effect at the level of ASM activation

Collectively, the force potentiation of one spasmogen (or baseline tone) on another spasmogen can be due to many factors such as: 1-use of sub-threshold or barely active concentrations of spasmogens, which simply act additively in terms of ASM activation but will be perceived as being synergistic in terms of ASM-force, ASM shortening, airway narrowing or airway resistance; 2-use of geometry-dependent measurements such as airway resistance; so that the effect of the second spasmogen is augmented by the reduced airway caliber caused by the first spasmogen; 3-a synergistic effect due to the contribution of vascular, neural or inflammatory effects when tested *in vivo* or *in situ*, which can be observed when the spasmogens used have vasoactive and inflammatory activities or when they can trigger either a vagal reflex and/or an increase in the efferent cholinergic traffic; and 4-a true synergistic effect on myosin cross-bridge cycling due to the convergence of intracellular signaling pathways that are activated by the ligation of two distinct GPCRs (such as Ca^{2+} sensitization).

4.3.2 Force adaptation

There is an additional, more subtle way by which the tone can contribute to AHR. Recent studies from our laboratory have demonstrated that a short period (~30 min) of increased ASM-tone augments ASM strength over time (20, 21). Specifically, ASM was exposed to ACh and ASM-force generation in response to EFS was monitored both before and after the induction of the ACh-induced tone. The first EFS in the presence of tone was given 1 min post ACh administration and then at 5-min intervals thereafter. Addition of ACh immediately increased the total force (ACh-induced tone + EFS-induced force), suggesting that ASM generates more force in response to two sub-maximal stimuli (ACh + EFS) compared with EFS alone. More surprisingly, while the EFS-induced force decreased immediately following the administration of ACh, it recovered significantly over time. Since the tone produced by the exogenous ACh remained relatively constant, the total force increased over time. We termed this phenomenon 'force adaptation' (20). The potential significance of force adaptation on airway narrowing based on a computational model has been demonstrated recently (Bossé *et al*., in press in the journal of Respiratory Physiology & Neurobiology).

Force adaptation is distinct from the synergisms in force that were attributed to the convergence of intracellular signaling pathways described above, which occur as a result of activation of two distinct GPCRs. This form of synergism cannot occur when ACh is combined with activation of the ASM by EFS, which was the experimental setting that was used to describe force adaptation. This is because EFS triggers ASM contraction by releasing ACh from the nerve endings (145), which, in turn, binds on the same M_3 receptor as the exogenously administered ACh. In fact, all studies investigating the acute effect of a muscarinic agonist on EFS-induced force have failed to show a synergistic effect (57, 114). Therefore, despite abundant literature reporting synergistic effects between spasmogens on ASM strength, force adaptation is a phenomenon with no precedent.

The molecular mechanisms involved in force adaptation are also likely to be different from those described above because it occurs over minutes. This time-course would be too fast for

de novo gene expression; so it is not likely due to transcriptional activation of genes involved in ASM contraction induced by the initial tone. On the other hand, if it was due to biochemical events such as increased phosphorylation of MLC due MLCP deactivation leading to Ca^{2+} sensitization (as described in subsection 4.2.3), the potentiating effect would be very fast and seen at the first EFS following ACh administration (1 min). Taken together, the kinetic of force adaptation is too slow to be explained by fast biochemical reactions, such as protein phosphorylation, but too fast for *de novo* gene expression. We thus suggest that this time-dependent increase in ASM-force is due to post-translational mechanisms. All the necessary machinery responsible for this increased ASM-force might be present within the cell prior to activation, but it is only upon stimulation (i.e., induction of tone) that this protein machinery re-organizes to optimize generation of force. Processes such as actin (264) or myosin (213) filamentogenesis and/or re-arrangement of the cytoskeleton and its connectivity with the contractile apparatus, the plasmalemma and the ECM (264) are likely possibilities. However, these remain pure speculations as the mechanisms underlying force adaptation have never been investigated.

4.3.3 Increased tone on airway wall stiffness

Apart from ASM-force, increased ASM-tone can also impact other ASM contractile properties. For instance, stiffness is greatly enhanced upon ASM activation. By increasing ASM stiffness, an increased tone would reduce the strains experienced by the airway wall due to the cyclical stresses of breathing. Interestingly, Noble and coworkers (178) have demonstrated that the amount of airway wall strain induced by a DI is proportional to its bronchodilating effect (178). They also showed that the main factor limiting the strain was the magnitude of ASM-tone, which was controlled by delivering different concentrations of ACh. These observations suggested that the ASM needs not only to be stressed but to be stretched for a DI to be effective in reducing ASM contractility. They concluded by saying that a stiffer airway caused by an increased ASM-tone reduces the strain induced by the dynamic load associated with DI and, for this reason, reduces the bronchodilating effect of a DI.

Taken all together, these observations suggest that baseline tone is of crucial importance in determining the degree of airway responsiveness to an inhalational challenge with a spasmogen. The increased tone observed in asthma can be from inflammatory and/or vagal origin. In both cases, this increased tone acts additively with the extrinsically delivered spamogen in terms of ASM activation, force generation, ASM shortening and airway diameter narrowing. This can also be translated into a synergistic effect when geometry-dependent measurements are taken, such as airway cross-sectional area of the lumen or airflow resistance (or other measurements relying on it, such as peak expiratory flow (PEF), FEV_1, …). In cases where the increased tone is mediated by inflammation-derived spasmogens, the potentiating effect of increased tone on airway responsiveness can also be due to a vascular effect, a neural effect, an inflammatory effect and/or an increase in ASM-force caused by either Ca^{2+} sensitization or force adaptation. Finally, augmented tone also increases ASM stiffness and, concomitantly, airway wall stiffness, which can further enhance airway responsiveness by many ways as discussed in subsections 2.2 and 2.3.

4.4 Heterogeneity

To assess airway responsiveness airway resistance is measured at the mouth using the forced oscillation technique or, alternatively, flow parameters are measured using standard spirometry. These measurements take into account airway resistance but also lung tissue

and chest wall resistance. Since chest wall resistance does not change during bronchoprovocation, and lung tissue resistance does not change by much, the progressive increase of resistance occurring in response to increasing doses of a spasmogen is thought to reflect changes of airway caliber. In other words, the change of resistance along the course of a challenge is viewed as being due to airway narrowing caused by ASM shortening. However, even this change of resistance is intricate. This is because this resistance represents represents the combined resistance of all generations of airways arranged in series and in parallel. Therefore, simply looking at the factors affecting resistance in a single airway, as we have done since the beginning of this chapter, may not be sufficient to understand the full nature of airway responsiveness.

4.4.1 Mathematical link between airway narrowing heterogeneity and airway responsiveness

The link between heterogeneity and AHR was initially though as being simply due to geometric considerations. As aforementioned, the relationship between airway luminal radius and resistance to airflow is not linear. A decrease in radius causes an exponential increase in airway resistance. For this reason, even if the total cross sectional area within a given airway generation is the same, inhomogeneous airway caliber increases airway resistance. In other words, the increase in resistance caused by narrowing of some airways is not compensated for by the decrease in resistance caused by dilation of other airways. Airway narrowing heterogeneity upon a bronchoprovocative challenge with a spasmogen will have the same consequence. So that the increases in resistance caused by augmented narrowing in some airways are not compensated for by the attenuated increases in resistance caused by reduced narrowing happening in other airways.

The consequences of baseline heterogeneity on baseline resistance and of non-homogeneous airway narrowing on airway responsiveness were predicted based on computational models. Bates (14) was one of the first to address the issue of heterogeneity. He developed a stochastic model of the airway tree in which the values of airway radii within every airway generation were chosen randomly according to a probability distribution function. The mean values were based on realistic values (morphometric data of human lungs). He found that, while the mean values were kept the same, increasing the standard deviations progressively leads to greater airway resistance. This suggested that the simple presence of heterogeneity increases airway resistance. The model further predicted that, upon ASM activation, both baseline airway caliber heterogeneity and ASM shortening heterogeneity increase airway responsiveness. Obviously, this is only true if one assumes that the flow is not redistributed into more patent airways.

More sophisticated computational models have then been developed and brought additional insights about the origin of this increased resistance evoked by baseline or airway narrowing heterogeneity. The main contributor to this increased resistance comes from nearly closed peripheral airways, especially when resistance is measured at frequency near the breathing frequency (138). These airway closures (or near closed) ultimately lead to ventilation defects and attendant flow limitation. They might be the link between ventilation heterogeneity and AHR.

4.4.2 Non-uniform ventilation with air trapping and AHR

Using the forced oscillation technique, several groups noticed that the frequency-dependence of resistance and elastance was increased at baseline in asthmatics (139) and

upon MCh challenge in both asthmatics and non-asthmatics (111, 139). In accordance to the computational models discussed above, this is indicative of a heterogenous pattern of constriction that includes randomly distributed airway closures or near closures (138). These observations are supported by ventilation imaging studies such as hyperpolarized ^{3}He MRI (39, 207, 234), single-photon emission computed tomography (116) and positron emission tomography (169). These studies showed that asthmatics have increased ventilation heterogeneity at baseline and, even if heterogeneous airway response to a spamogen is also observed in non-asthmatics, the level of ventilation heterogeneity achieved is greater in asthmatics (39, 207, 234). This heterogeneous pattern of ventilation is characterized by relatively large zone of non-ventilated area, which supports the presence of airway closure. These ventilations defects represent zones of air trapping, which are characteristic of obstructive diseases and known to contribute to airflow limitation. Ventilation heterogeneity can thus be one of the sources of AHR seen in asthmatics. This is consistent with recent reports showing that baseline ventilation heterogeneity measured by nitrogen washouts correlates with the severity of AHR in both younger (60) and older (96) asthmatic subjects.

The increase in ventilation heterogeneity in response to a spasmogenic challenge may simply be due to an amplifying effect over the baseline heterogeneity. However, upon induced bronchoconstriction, airway narrowing heterogeneity can also contribute to ventilation heterogenetity. King and coworkers (115) have used high resolution computed tomography (HRCT) to measure the heterogeneity of airway narrowing in response to MCh challenge in human subjects. The heterogeneity was measured by the standard deviations of the changes in luminal airway caliber caused by MCh. They showed that in airways of ≥ 2 mm of diameter that the variability of airway narrowing was greater than the measure of repeatability (the variability in the changes in luminal airway caliber when two pre-MCh scans were compared), suggesting that airways narrow heterogeneously. They also shown that for the same level of bronchoconstriction achieved, asthmatic airways narrowed more heterogeneously than non-asthmatics. Together, these observations suggest that the larger and more numerous ventilation defects observed in asthmatics during a spasmogenic challenge are due to both greater baseline heterogeneity and greater airway narrowing heterogeneity.

4.4.3 Mechanistic link between airway narrowing heterogeneity and airway responsiveness

As aforementioned, the emergence of ventilation defects during bronchoconstriction is essentially the link between ventilation heterogeneity and AHR. In other words, simple heterogeneity without airway closure (or near closure) does not seem to cause AHR. It is true that for the same mean level of bronchoconstriction, heterogeneity would increase resistance but only if the flow is still evenly distributed among the different airways. However, during heterogeneous constriction, the flow is more likely to be redirected into more conductant (patent) airways and correspondingly less flow would be redirected into more resistant (constricted) airways. Consequently, the overall resistance would rather decrease with heterogeneity. On the other hand, when the airways are closed (or nearly), the volume of air subtended by these airways is trapped and will not be able to be expelled (at least not as fast) upon maximal expiratory flow maneuvers and that would be the cause of AHR.

It is also unlikely that the ventilation defects are due to random closure of single peripheral airways. Based on the size of these ventilation defects, they are more likely due to clustering

of constricted peripheral airways. In a computational model developed by Venegas and coworkers (236, 253), it was shown that airway closure occurs in cluster. In their model, the bronchial tree branches dichotomously, where every bifurcation is composed of one parent airway and two daughter airways with slight heterogeneity between them. During bronchoconstriction, the flow is redistributed according to divergence of patency among daughter airways. The model predicted that all the airways shorten uniformly when the level of ASM activation is low. However, passed a certain threshold of ASM activation, daughter airways develop a dichotomic response. Whereas one constricts excessively the other one dilates. This is because the initial heterogeneity between the two daughter airways fosters the redistribution of flow into the more conductant airway. The insufflation pressure thus rises in the hyperventilated areas subtended by this airway, which increases the load impeding ASM shortening. In fact, the model predicted that this airway dilates despite the rise in ASM activation. On contrary, diminution of flow in the other daughter airway causes regional lung deflation and loss of parenchymal tethering recoil. The load impeding muscle shortening thus decreases and, for the same level of ASM activation, more narrowing occurs. Because of axial interdependence of pathways in series, all the smaller airways downstream of the excessively closed airways are also affected. The size of the ventilation defects depends on the first airway generation afflicted by this dichotomic response. Since more ASM activation is required to close larger airways, this dichotomic response begins in more peripheral airways. But as the ASM activation progressively rises, the model predicted that larger and larger airways are affected and larger and larger patches of lung become non-ventilated.

Taken together, Venegas and colleagues suggested that only a slight degree of heterogeneity at baseline can trigger a self-perpetuating feedback loop when a certain level of ASM activation is achieved; such that the redistribution of flow in slightly more patent airways, makes the other airways and their downstream pathways unstable because of the loss of elastic recoil. This ultimately leads to clustering of peripheral airway constriction and the emergence of large ventilation defects, which, in turn, cause flow limitation. The chase for the factors determining this baseline heterogeneity, which was heretofore ignored, is now on.

4.4.4 Factors potentially involved in determining baseline heterogeneity

The amplifying effect described by Venegas and coworkers (236), which can lead to large ventilation defects and attendant AHR, relies on subtle structural and/or functional changes that were already present prior the inhalation of the spasmogen. Therefore, the identification of factors responsible for this baseline airway wall heterogeneity is of major interest.

Remodeling can certainly impact heterogeneity and can potentially discriminate the different levels of heterogeneity observed between asthmatics and non-asthmatics. Remodeling occurs non-uniformly throughout the tracheobronchial tree. The occurrence of epithelium desquamation or inflammatory infiltrates for example is patchy in nature. The extent of subepithelial fibrosis, ASM enlargement, and other structural changes present in asthmatic lungs could also vary at different locations in the lung. Together, these disparities alter the mechanical properties of the airway wall non-uniformly along the bronchial tree. It can also modify airway geometry and sometimes lead to the formation of bottlenecks, a characteristic feature found in terminal bronchioles of chronic obstructive pulmonary disease (COPD) patients (McDonough *et al*., provisionally accepted in the New England Journal of Medicine). The effect of a bottleneck is treacherous because not only it increases the resistance

to airflow in an airway that otherwise may look normal but also because it reduces ventilation in the parenchymal tissue subtended by this airway. This ultimately fosters the development of a ventilation defect due to a decrease in the force of airway-parenchymal interdependence. Greater ventilation defects at baseline seen in asthmatics can thus be attributed to remodeling.
However, ventilation heterogeneity is mainly attributable to the fact that airways narrow heterogeneously. So, it is upon bronchoconstriction that the functional consequences of these disparities (localized remodeling changes) along the length of a pathway are exacerbated. For example, remodeling heterogeneity can affect locally the initial geometry, which has a huge influence on airway responsiveness (as discussed in subsection 4.1.3). It can also modify locally the after-load impeding ASM shortening. Another example would be the patchiness of ASM mass enlargement, which can potentially amplify regional differences in airway narrowing upon ASM activation.
Gain in contractile properties (e.g., force-generating capacity) due to ASM behaviors could also be patchy within the lungs. For example, acquisition of supplemental force by length adaptation can occur in an area where the airway caliber is smaller because of a localized decrease in the force of airway-parenchymal interdependence. The baseline ASM-tone also likely differs in different areas of the lung. Its magnitude may be spatially correlated with zones of inflammation, where inflammation-derived spasmogens are produced/released. That would also lead to force adaptation and the attendant gain in force-generating capacity in those areas. All these factors can affect ASM contractile properties in a localized manner and contribute to narrowing heterogeneity and AHR.
Hence, remodeling and inflammatory changes seen in asthmatic lungs can give rise to a greater baseline level of airway wall heterogeneity. It can also cause inhomogeneity of narrowing upon a bronchoprovocative challenge with a spasmogen by changing the load impeding ASM shortening or by fostering the development of increased ASM contractility. Together with the strong interplay between the parenchymal tethering and airway patency, this increased level of heterogeneity can be sufficient to trigger a self-perpetuating loop leading to patches of hypo- or non-ventilated area and the appearance of AHR in asthmatics.

5. Conclusion

Despite abundant evidence implicating the ASM as the culprit of AHR in asthma, the latest genetic studies are relatively weak to support the role of asthma susceptibility gene risk variants in causing ASM dysfunction. The more reliable genetic analyses performed to date have instead suggested that the well-recognized genetic predisposition to suffer from asthma may be related to polymorphisms in genes that are predominately involved in immunology and/or epithelial integrity and functions. Even if we cannot rule out the contribution of genetic alterations affecting the ASM in AHR at the present time, the bulk of evidence rather suggests that if alterations in ASM mechanics contribute to AHR, these alterations are acquired as a result of inflammatory mediators and/or extracellular matrix remodeling that are present in asthmatic lungs. For example, lung defects can foster the appearance of normal ASM behaviors that render the muscle hypercontractile, such as length adaptation, force adaptation and changes in ASM cell's phenotype. These ASM behaviors are the testimony that he contractile properties of the ASM are not fixed but rather plastic (adaptable).
On the other hand, alterations in ASM contractile properties are not necessarily a prerequisite to suffer from AHR. Many lung defects are sufficient alone (i.e., without the

need of increased ASM contractility) to cause AHR. These include but are not restricted to: 1-alterations in epithelial integrity, which increase the bioavailability of the inhaled spasmogen onto the ASM; 2-increased ASM-tone (due to the preponderance of inflammation-derived spasmogens, an augmented vagal input and/or decreased expression of relaxant factors), which acts additively with the extrinsically-delivered spasmogen in terms of ASM activation and ASM shortening, and synergistically in terms of narrowing of the cross-sectional area of the lumen and airway resistance; 3-obstruction of the vessels irrigating the ASM, which prevents clearance of the spasmogens into the circulation and concomitantly sequesters the spasmogens in the vicinity of ASM; 4-increased mass of the material inner the ASM layer, which increases airway narrowing for any given degree of ASM shortening; 5-decreased ASM-load (due to adventitial thickening, detachment of the parenchymal tethers from the outer edge of the airway wall, decrease in lung recoil, reduction in lung volume, diminution of the radial constraint limiting ASM bulging during shortening, other changes in ECM constitution which may change the mechanical properties of the airway wall to make it easier to compress by affecting, or not, its pattern of folding), which increases ASM shortening for any given degree of ASM activation; and 6-ventilation heterogeneity with airway closure, which affects flow measurements (such as FEV_1, PEF and others) because of air trapping. For all of these reasons, some asthmatics can be hypperresponsive even when their ASM operates as normal. In conclusion, we think that the ASM is often 'blamed' for the AHR seen in asthma simply because of its unequivocal role in airway responsiveness. We would like to propose that without further or more convincing proofs to incriminate ASM in AHR, this obeisant tissue should still be considered 'innocent'.

6. References

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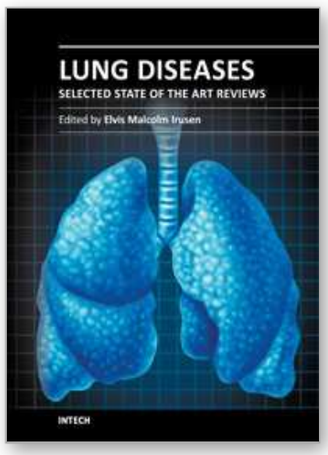

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