

Molecular mechanisms in allergy and clinical immunology

(Supported by a grant from Merck & Co, Inc, West Point, Pa)

Series editor: Lanny J. Rosenwasser, MD

Frozen objects: Small airways, big breaths, and asthma

Jeffrey J. Fredberg, PhD *Boston, Mass*

Airway hyperresponsiveness is one of the cardinal features of asthma but remains largely unexplained. The new concept of perturbed myosin binding within airway smooth muscle sheds light on the question of why airway narrowing is limited in the healthy lung and not in the asthmatic lung and points to unanticipated mechanisms through which lung development and allergic status may be major modulators of airway hyperresponsiveness. (*J Allergy Clin Immunol* 2000;106:615-24.)

Key words: Smooth muscle, myosin, inflammation, maturation, hyperresponsiveness, respiration

Airway hyperresponsiveness is the excessive narrowing of the airway lumen caused by stimuli that would cause little or no narrowing in the normal individual. It is one of the cardinal features of asthma but remains largely unexplained. Currently, much attention is being focused on the upstream events that initiate and then sustain the inflammatory response, with the expectation that improved understanding of these events will help to illuminate the causes of airway hyperresponsiveness. If these initiating events can be thought of as the ultimate cause of airway hyperresponsiveness, then this review turns attention to those factors that are at the most downstream end of the inflammatory cascade: the end-effector cell, airway smooth muscle, and the end-effector molecule, myosin II. These are the proximal agents of acute airway narrowing. This review examines an emerging synthesis that, to some extent, integrates ultimate and proximal causation and points to unanticipated mechanisms through which lung development and allergic status might be major modulators of airway hyperresponsiveness.

Before we climb down the reductionist ladder, I will lay out some of the mysteries of airway narrowing and the concepts that have been used to think about it in the past. I will then introduce the idea of perturbed myosin

Abbreviation used

ATP: Adenosine triphosphate

CSK: Cytoskeleton

binding and demonstrate the simplifications that arise when these mysteries are reexamined through that lens. Perturbed myosin binding by no means clears all the fog, but it does make connections between facts and ideas that had been considered to be unrelated and seemingly inexplicable. In doing so, it also helps to explain why airway hyperresponsiveness has been such a perplexing phenomenon.

THE UNBEARABLE LIGHTNESS OF BREATHING

Airway smooth muscle in the lungs of healthy animals possesses sufficient force-generating capacity to close all airways.^{1,2} This fact may at first seem to be unremarkable, but it is not easily reconciled with other observations. When healthy animals or humans are challenged with nonspecific contractile agonists in concentrations thought to be sufficient to activate the muscle maximally, resulting airway narrowing is limited in extent, and that limit falls far short of airway closure.³⁻⁵ Breathing remains unaccountably easy. Indeed, it is this lightness of breathing in the healthy challenged lung, rather than the labored breathing that is characteristic of the asthmatic lung, that in many ways presents the greater challenge to our understanding. It has been suspected for some time that some unidentified mechanism must act to limit the extent of airway smooth muscle shortening in the healthy lung but becomes compromised in the asthmatic lung. Furthermore, it has been suspected that the impairment of that mechanism, if it could only be understood, might help to unlock some of the secrets surrounding excessive airway narrowing in asthma, as well as the morbidity and mortality associated with the disease.

OLD IDEAS: HOMEOSTASIS, EQUILIBRIUM, AND STABILITY

The classical theory of airway lumen narrowing was developed to explain the determinants of airway narrow-

From the Department of Environmental Health, Harvard School of Public Health, Boston.

Received for publication May 8, 2000; revised June 12, 2000; accepted for publication June 14, 2000.

Reprint requests: Jeffrey J. Fredberg, PhD, the Department of Environmental Health, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/109429

doi:10.1067/mai.2000.109429

ing and why that narrowing can become excessive in patients with asthma. The classical theory emphasizes that muscle length and airway caliber are set by a force balance in which the active force generated by airway smooth muscle statically is in mechanical equilibrium with the passive reaction force developed by the elastic load against which that muscle has shortened. Because both forces depend on muscle length, the muscle accommodates itself to the length at which these opposing forces come into static balance.⁶⁻⁹ The key ideas here are the force balance and its static nature: the bigger the active force or smaller the elastic load, the smaller the equilibrium caliber of the airway lumen. The isometric force-generating capacity of airway smooth muscle is set principally by muscle mass, muscle contractility, and muscle position on its static force-length characteristic.^{7,10,11} Taken together, evidence available in the literature suggests that there is no systematic difference in force-generating capacity between muscle from the normal versus the asthmatic lung, although this evidence is rather equivocal.¹²⁻¹⁴ The passive reaction force against which the muscle shortens is set principally by the elasticity and geometry of the airway wall, tethering of the airway to the lung parenchyma, and the state of lung inflation.^{3,15-19} Each of these factors, in turn, have their own determinants that are known to be modified with chronic airway inflammation.^{9,20-22}

This balance of static forces is sustained by cyclic interactions of myosin with actin. With onset of the contractile event, myosin-actin cycling begins, and the number of interactions (bridges) increases and eventually approaches a steady state. It is widely agreed that during this process rapidly cycling cross bridges are replaced progressively by slowly cycling latch bridges if given enough time at a fixed muscle length, but the mechanisms of cycling rate regulation remain very much an open question in the literature.²³⁻³¹ Among the regulatory mechanisms that have been proposed, the latch scheme of Hai and Murphy²³ has the attributes of being the simplest and capturing the central importance of phosphorylation of the 20-kd myosin regulatory light chain. Within the latch schema, the attainment of the isometric steady state implies that the population distribution of myosin molecules among their 4 possible states (attached vs unattached to actin and phosphorylated vs unphosphorylated regulatory light chains) have come to a binding equilibrium set by a balance of kinetic rate processes, many of which are adenosine triphosphate (ATP) dependent. Once enough time has passed that this balance is attained and myosin has come to a binding equilibrium appropriate to isometric steady-state conditions, the muscle is then said to be in the latch state. Thus the latch state corresponds to what I will refer to as a static equilibrium of myosin binding.

These classical concepts of a balance of static forces at the mechanical level and a static binding equilibrium at the molecular level have explained much of what needed to be explained about airway lumen narrowing in asthma.^{6,7,11} These ideas have formed the foundation of our

understanding of airway lumen narrowing in the healthy and the asthmatic subject and have proven to be useful because they define the static equilibrium length toward which activated airway smooth muscle would tend if given enough time. New evidence now shows, however, that this classical point of view is fundamentally wrong and, in realistic physiologic circumstances, fails even to provide a reasonable approximation.³² The reason for this failure is that the classical view limits itself to equilibrium considerations and static factors, whereas regulation of airway smooth muscle length is now known to be a nonequilibrium process that is fundamentally dynamic. Under nonequilibrium conditions, the airway does not have to follow the expected behavior of equilibrium systems. The old view of homeostasis, stability, and equilibrium gives way to a dynamic world of fluctuation, instability, and nonequilibrium behavior.

FLIRTING WITH DISASTER

Smooth muscle surrounding the airway shortens when it is activated, and as the muscle shortens, airway narrows and breathing tends to become labored. The lung has a potent built-in defense against bronchospasm, however, and this defense works the other way around: the very act of breathing makes it difficult for activated muscle to shorten.³³⁻⁴⁰ Asthma is an inflammatory disease, but could it be that it is the failure of this particular defense mechanism that is the most telling end effect of the inflammatory cascade and therefore the proximal cause of airway hyperresponsiveness in asthma? This idea is not at all new,³³ but the emergence of new evidence and new understanding of underlying mechanism invites reconsideration of the question.

We breathe all the time, and we sigh at the rate of about 10 times per hour.⁴¹ The bronchodilating effect of this pattern of breathing is so effective that airway narrowing never approaches dangerous levels in healthy people, even when challenged with high concentrations of non-specific bronchoconstricting agents. We can put the potency of this mechanism into perspective with the following observations. The expected physiologic range of tidal muscle stretch is from about 4% of muscle length during spontaneous breathing at rest to 12% during a sigh and greater still during exercise. In isolated activated muscle, however, tidal stretches of only 3% of muscle length are enough to inhibit active force generation by 50%.⁴² By contrast, rather high concentrations of isoproterenol (in the range of 10^{-6} mol/L or more) are required to attain, by purely pharmacologic means, the same degree of muscle relaxation caused by small tidal stretches that occur during breathing (A. Gump, unpublished observation). Moreover, in healthy volunteers who inhale bronchoconstricting substances, such as histamine, there is a reflex increase in the frequency and depth of spontaneous sighs when bronchospasm begins, and these deep inspirations cause prompt and nearly complete dilation of the airway.^{36,43,44} Even when healthy volunteers inhale some of the most potent known bronchoconstrictors, such as

leukotrienes, bronchospasm is profoundly blunted unless deep inspirations are prohibited.⁴⁵ Taking into account the levels at which endogenous dilators are found in the airway, these observations suggest that the tidal muscle stretches that are attendant to spontaneous breathing comprise the first line of defense against bronchospasm and that tidal muscle stretches may be the most potent of all known bronchodilating agencies.

During an asthmatic attack, this bronchodilating mechanism fails. Indeed, there is ample evidence from the work of Ingram et al⁴³ to show that, if anything, deep inspirations only serve to make matters worse during an asthmatic attack. In this connection experiments conducted years ago led Fish et al³³ to the striking observation that airway obstruction in asthma behaves as if it was caused by an intrinsic impairment of the bronchodilating effect of a deep inspiration, as opposed to an inappropriate end responsiveness of the airway itself. At about the same time, similar observations led Orehek et al³⁶ to speculate that asthma triggers a vicious cycle in which asthmatic airway obstruction increases the frequency of deep inspirations, and deep inspirations, in turn, make the obstruction worse.

This impairment of the bronchodilating effect of a deep inspiration was long thought to be a characteristic of only spontaneous asthmatic obstruction and the late-phase response to allergen challenge.^{43,46} Therefore it came as a surprise to learn only recently that an impairment of this kind is easily evoked in completely healthy individuals. Two laboratories have shown that if healthy, nonasthmatic, nonallergic subjects do nothing more than to voluntarily refrain from deep inspirations but otherwise maintain normal tidal volume, minute ventilation, and functional residual capacity, within 15 minutes their airways become hyperresponsive to a degree that is virtually indistinguishable from that observed in asthmatic subjects.^{35,47,48} Even more remarkable, when deep inspirations are eventually reinstated, the subsequent ability of deep inspirations to dilate the airways becomes profoundly impaired, just as it does in spontaneous asthmatic obstruction. Put simply, it is as if the airway smooth muscle, when activated, is all the time flirting with disaster, and the mere removal of deep inspirations, which would seem superficially to be a rather trivial matter, is sufficient nonetheless to precipitate a cascade of events that is much more serious, even in healthy volunteers with no airway inflammation, no history of airway inflammation or allergy, and airways and airway smooth muscle that are perfectly normal.

STUCK ON LATCH

How is all this to be explained? The theory of perturbed myosin binding was put forward only recently and suggests that the tidal action of lung inflations may play a pivotal role in myosin dynamics.³² Lung inflations strain airway smooth muscle with each breath, and these periodic mechanical strains are transmitted to the myosin head and cause it to detach from the actin filament much

sooner than it would have in isometric circumstances; myosin binding is strongly perturbed by the action of breathing.⁴⁹ This premature detachment profoundly reduces the duty cycle of myosin, typically by as much as 50% to 80% of its isometric (ie, unperturbed) steady-state value and depresses total numbers of bridges attached and active force to a similar extent. Therefore of the full isometric force-generating capacity of the muscle, only a small fraction ever comes to bear on the airway during tidal breathing, even when the muscle is activated maximally. In pathologic circumstances, however, the tidal strains acting on myosin can become compromised. For example, in the chronically inflamed airway the peribronchial adventitia thickens.⁵⁰ This thickening unloads the muscle, decreases tidal muscle strains, and in doing so sets off a sequence of events (described below) that permit myosin binding to approach an equilibrium applicable to static conditions. The muscle would then generate the full complement of isometric force appropriate to the stimulus and can shorten to a point approaching airway closure. Perturbed myosin binding seems to be the best theory available for understanding why airway narrowing is limited in the healthy lung and not in the asthmatic lung, although, as described below, it is a very imperfect explanation.

When myosin binding is perturbed, the magnitude of the contractile response becomes functionally disengaged from the level of the contractile stimulus.³² As a result, the muscle can stay compliant and long, even when the level of muscle stimulation is supramaximal. Breathing is good for breathing, and breathing punctuated by occasional deep inspirations is better still. This is because the first bridge to attach, working alone, is easy to break, and so on for the next and the next. As they pop up one by one, breathing mows them down. Early in the contractile event, breaking incipient bridge activity is easy, and this keeps the muscle compliant and blunts the contractile response, keeping it in check. In the steady state the process becomes dynamically equilibrated, and only a small fraction of the bridges that can attach are attached (Fig 1, *bottom*). Putting it another way, if actomyosin is the molecular motor through which metabolic energy is converted into the mechanical energy that drives airway narrowing during bronchospasm, then the tidal action of breathing works just the other way around. It uses mechanical energy supplied by the muscles of the chest wall to disrupt those same molecular events, thereby hoisting smooth muscle actomyosin on its own petard. Recent evidence suggests that it may be this dynamically equilibrated state of affairs that limits the extent of narrowing in the normal airway and that the normal pattern of spontaneous breathing is sufficient to maintain it,^{32,35,39,47,48} although just barely.

This virtuous spiral of self-reinforcing events seems to be rather precarious and can unravel or collapse on itself if the amplitude of the force fluctuations that stretch the muscle should somehow become compromised. For example, force fluctuations are linked intimately to peribronchial stress, distending the airway and its changes in

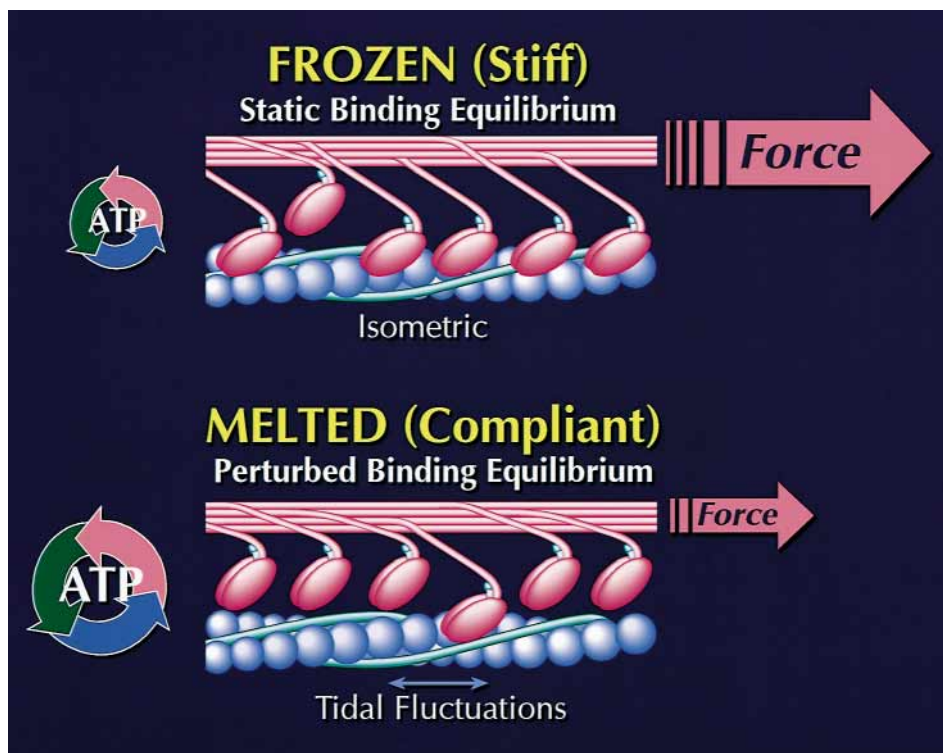


FIG 1. *Top*, In isometric steady-state contraction myosin comes to a binding equilibrium corresponding to static conditions. The duty cycle of the myosin cross-links is high, and at any instant a large fraction of the links that can attach to actin are attached. As such, this state, which is called the latch state, is characterized by high muscle force and high muscle stiffness. The interactions of myosin with actin are cyclic and ATP dependent, although in the isometric steady state the cycling rate is very slow. The latch state approximates a frozen state of the muscle because the muscle is very stiff, there are many links attached, they are cycling very slowly, and the rate of ATP use (per unit force maintained) is very small. *Bottom*, When subjected to load fluctuations, as occurs during breathing, the actin filament will undergo small periodic axial displacements (*horizontal arrow*) relative to the myosin backbone. These displacements perturb the binding of myosin to actin by causing the myosin to detach much sooner that it would in an isometric contraction. As a result, only a small fraction of the links that can attach to actin are attached. This perturbed state is characterized by small muscle force and approximates a melted state because the muscle is compliant, there are few links attached, they are cycling very rapidly, and the rate of ATP use (per unit force maintained) is high.

time. Any factor that lessens peribronchial stress will decrease the force fluctuations impinging on the muscle, including inflammatory thickening of the peribronchial adventitia, loss of lung elastic recoil, breathing at low lung volumes, and failure to take deep breaths.^{3,6,7,9,51} If so, then with each breath the muscle would stretch a bit less, but if it stretches less, the muscle will become stiffer still. This is because many attached bridges working in parallel are harder to break. As the contraction develops, ultimately the muscle can become so stiff that the physiologic forces acting on the muscle find themselves to be quite insufficient to stretch the muscle appreciably, leaving the muscle so stiff that it becomes virtually frozen and stuck at its static equilibrium length.³² The muscle could be said to be frozen in the latch state (Fig 1, *top*). Thus bronchial hyperresponsiveness, the hypothesis suggests, would correspond to failure of the underlying perturbed equilibrium to sustain itself and an ensuing return of myosin to the binding equilibrium that pertains in static conditions and

latch. Because the perturbed equilibrium of myosin binding inhibits shortening of activated muscle, the collapse of this dynamic system to static equilibrium conditions would represent the disinhibition of that process.

PERTURBED EQUILIBRIA: NECESSARY BUT NOT SUFFICIENT

When maximally activated muscle in the muscle bath is subjected to progressively increasing load fluctuations approaching the magnitude and frequency expected during normal breathing, the muscle lengthens appreciably in response. This phenomenon is called fluctuation-driven lengthening, and it is accounted for quite well by perturbed equilibria of myosin binding.³² When load fluctuations are progressively reduced, the muscle reshortens somewhat but fails to return to its original length. Incomplete reshortening after exposure to tidal loading is not accounted for by muscle injury or fatigue; the original

operating length can be recovered simply by removing the contractile agonist and allowing the muscle a short interval before recontracting. Incomplete reshortening cannot be accounted for by myosin dynamics either because myosin dynamics by themselves predict complete reshortening when the load fluctuations are removed.³² Thus the failure of activated muscle to reshorten completely is evidence of a remarkable plasticity of the contractile response. During a sustained contraction, the operational length of the muscle can be reset by loading and the history of that loading.^{32,52,53}

This newly discovered plasticity of the contractile response would appear to be an important bronchodilating mechanism because it shows us that a series of deep inspirations early on can reset muscle length and airway caliber to larger values on a semipermanent basis (ie, for the remainder of the contractile episode). However, the mechanism of this plasticity is unknown, although a clue comes from an unexpected quarter: the work of Pierre deGennes, a recent awardee of the Nobel Prize for his work in polymer physics.

FROZEN OBJECTS

As strange as it may seem, the fluctuation-driven changes of state described above conform rather precisely to the conceptual framework of the nonequilibrium phase transition (ie, melting and freezing), as described in general terms by Prigogine and Lefever⁵⁴ and Nicolis and Prigogine⁵⁵ and in weak gels subjected to finite stresses by deGennes.⁵⁶ In all materials phase transitions are driven at the molecular level by force fluctuations, which can arise either from innate thermal agitation and its changes with temperature (the familiar equilibrium phase transition, like the melting of ice) or from external force fluctuations applied at the system boundary (the less familiar nonequilibrium phase transition). This latter case may apply to airway smooth muscle in the lung during breathing. It would seem to be not only convenient but also correct therefore to think of the contractile state associated with negligible load fluctuations as a frozen or solid-like molecular state because, in that circumstance, the muscle has many myosin cross-links attached at any moment and the underlying rate of turnover of those links is relatively slow (Fig 1, *top*). This is the latch state, a cold molecular state characterized by high muscle stiffness, slow bridge cycling, and a relatively low rate of specific ATP metabolism.⁵⁷ When this cold molecular state prevails, the physiologic expectation would be one of high muscle forces, excessive airway narrowing, inability of deep inspirations to stretch the muscle (because of its high stiffness and associated inability to perturb myosin-actin bonds), and a transient constrictor response to a deep inspiration (because of the low hysteresis of muscle in the frozen state relative to that of the surrounding lung parenchyma).^{43,57,58} Remarkably, all of these expectations are in fact prominent characteristics of spontaneous airway obstruction in asthma that previously had been unexplained. Likewise, it is useful to think of the con-

tractile state associated with larger load fluctuations (in the range typical of quiet tidal breathing) as a melted or liquid-like state; here, myosin binding is strongly perturbed, with few links attached at any moment but turning over rapidly (Fig 1, *bottom*). This is a hot molecular state characterized by small muscle stiffness, rapid bridge cycling, and a relatively high rate of specific ATP metabolism.³² When this hot molecular state prevails, the physiologic expectation would be one of lower muscle forces, limited airway narrowing, the ability of deep inspirations to stretch the muscle, and a corresponding transient dilatory response to a deep inspiration (both caused by breaking myosin links and high hysteresis of muscle in the melted state relative to that of the lung parenchyma). These expectations on the basis of perturbed myosin binding are in fact known to be characteristic of airway obstruction induced by exposure to non-specific contractile agonists. There is now a growing body of evidence to suggest that load fluctuations in the physiologic range are big enough that they can break through myosin-actin cross-links and, in effect, melt the contractile domain.

Myosin is the primary effector molecule of the contractile response, but this motor protein exerts its mechanical effects within a cytoskeletal scaffolding that is both deformable and in a continuous state of remodeling. The speculation, then, is that secondary but important molecules stabilize the CSK, and as the contractile domain melts under the influence of imposed load fluctuations, those loads must be borne increasingly by the scaffolding itself.^{59,60} Alternatively, Pratusевич et al⁵³ have suggested that the architecture of the myosin fibers themselves may change, whereas Gunst et al⁵² have argued that it is the connection of the actin filament to the focal adhesion plaque at the cell boundary that is influenced by loading history. Regardless of the specific molecules and mechanism invoked to explain the plasticity of the contractile response, the melting of the contractile domain would appear to be a necessary (or permissive) event but one that by itself is not sufficient to explain the effects of the history of tidal loading.

HYPERRESPONSIVENESS = ACTIVATION + UNLOADING

During breathing, airway smooth muscle contracts against a load that possesses both a steady and a fluctuating component. It is now well established that in response to nonspecific contractile agonists, greater airway narrowing occurs if either the steady (static) component of the load is decreased³ or if the fluctuating component of the load is decreased.^{2,4,39,61} If the steady and fluctuation components of the load should become compromised at once, as is the case in the situations described below, the muscle experiences a double whammy, and the shortening of activated muscle would be expected to be extensive. For example, consider what happens when the peribronchial adventitia undergoes cytokine-driven inflammatory thickening,^{11,21,50,62} when the lung loses elastic

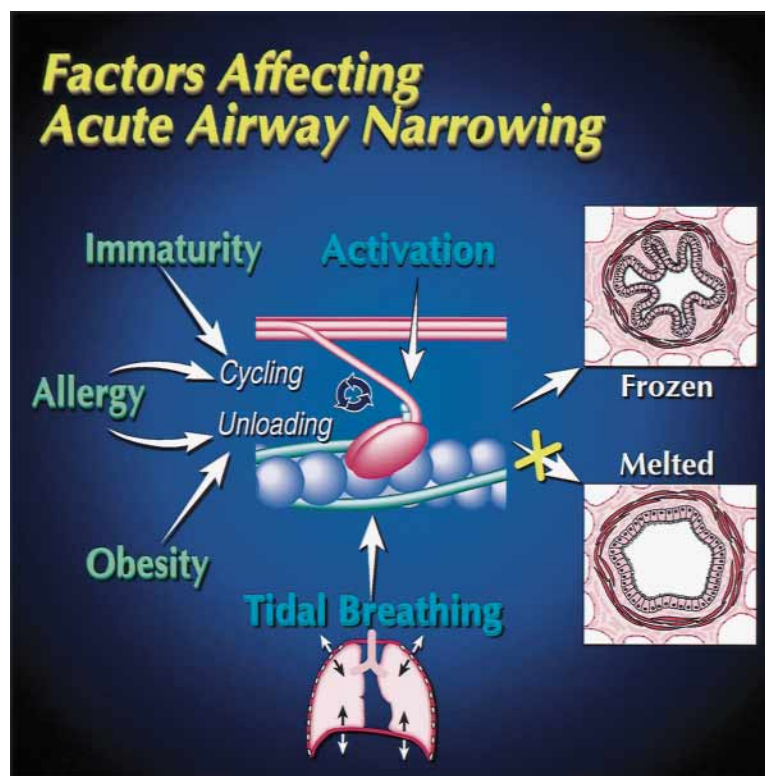


FIG 2. The cyclic interaction of myosin with actin is activated by intracellular calcium but is modulated by factors that regulate myosin cycling (including allergy and immaturity) and myosin loading (airway remodeling and obesity). In normal circumstances the effects of tidal breathing are sufficient to perturb myosin binding and maintain maximally activated muscle in the melted state. In that case acute airway narrowing is limited, and airway caliber is controlled by a dynamically equilibrated nonequilibrium process. However, any number of factors can destabilize the system, block the melted pathway, and cause the system to collapse to a static binding equilibrium, in which case the airway smooth muscle will shorten and freeze (see Fig 1). In that case airway smooth muscle has sufficient force-generating capacity to close every airway in the lung and will become refractory to the beneficial effects of deep inspirations.

recoil, or when tidal lung expansion becomes diminished. In each of these cases both the steady and the fluctuating component of the muscle load become compromised (Fig 2). These instances bring immediately to mind not only asthma but also emphysema, normative aging, restrictive disorders of the chest wall, obesity, and cervical spinal cord injury (Fig 3). Each of these situations is known to be associated with a predisposition for airway hyperresponsiveness.⁶³⁻⁶⁷ Unloading of the airway smooth muscle may come into play similarly in nocturnal asthma because both tidal volume and functional residual capacity fall during sleep.^{68,69} Moreover, it is clear that when inflammatory remodeling of the airway does occur, the perturbed equilibrium hypothesis predicts that the resulting predisposition for airway hyperresponsiveness might persist long after the inflammation itself is resolved.^{70,71} Finally, perturbed equilibria might also help to explain why the obstructive response in exercise-induced asthma (hyperpnea-induced bronchospasm) typically begins only after cessation of the exercise, when tidal volumes have declined to resting levels.

MYOSIN: THE STORY OF A GOOD MOLECULE GONE BAD?

We see then that in the right mechanical environment the action of the myosin motor may be rather modest, even when contractile activation is supramaximal, but as noted earlier, in the wrong mechanical environment (ie, in a static environment), even normal airway smooth muscle has the capacity to close every airway in the lung. The airway smooth muscle itself might be perfectly normal, but airway hyperresponsiveness might arise nonetheless. Excessive shortening of the muscle on activation would be attributable not to the muscle itself but rather to the mechanical environment in which it operates.

Even if the force-generating capacity of the muscle is normal, there may yet be a problem with the muscle after all. The problem, though, may not be that it is too strong but rather that it is too fast. Stephens and colleagues^{13,72-74} have noted an association between shortening velocity and airway hyperresponsiveness, even when the force-generating capacity of the muscle was the same. Howev-

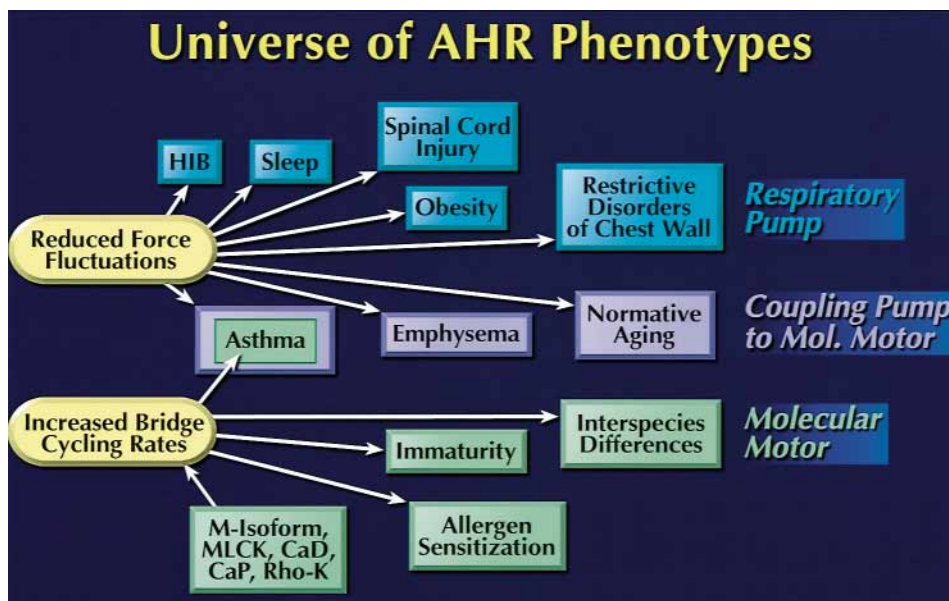


FIG 3. The perturbed equilibrium hypothesis is attractive because it is rather simple yet seems to stitch together within a unified framework a diverse group of airway hyperresponsiveness phenotypes that were largely unexplained and had been thought to be essentially unrelated. Reduced force fluctuations, increased bridge-cycling rates, or both allow airway smooth muscle to more nearly approach a static equilibrium of myosin binding (latch) and the frozen state. Airway hyperresponsiveness phenotypes shown in blue correspond to circumstances in which the airway and airway smooth muscle might be perfectly normal, but there is a problem with the respiratory pump (ie, the muscles of the chest wall). Those shown in purple correspond to phenotypes in which the airway smooth muscle may be normal, but there is a problem in the mechanical coupling between the respiratory pump and the myosin motor. Finally, those shown in green correspond to phenotypes in which the problem may be at the level of the myosin motor itself. At that level, the rate of bridge cycling is thought to be influenced by its isoform, the amount of myosin light chain kinase (*MLCK*), caldesmon (*CaD*), calponin (*CaP*), Rho-kinase (*Rho-K*), and other factors. *HIB*, Hyperpnea-induced bronchospasm.

er, how shortening velocity might relate to airway hyperresponsiveness was not clear. The argument to explain the connection and its implications is simple but subtle, although its validity has not yet been established experimentally.³² In brief it goes like this. In many, but not all, species, smooth muscle in the developing lung has a higher shortening velocity than in the mature lung.^{14,73} Higher shortening velocity implies faster myosin cycling, and this faster cycling has been attributed to differential expression of fast myosin isoforms earlier in life.⁷⁵ The faster the myosin cycling, the more difficult it is for imposed load fluctuations to perturb the actomyosin reaction because the faster the intrinsic rate of cycling, the faster will a bridge, once becoming detached, reattach and contribute once again to active force and stiffness. Because myosin cycling slows with lung maturation, the potent bronchodilation mechanisms associated with fluctuation-driven lengthening would be predicted to increase. Load fluctuations may be as potent a bronchodilator as the most potent pharmacologic agencies that we know of, but their effects would be expected to be compromised early in life.

An entirely parallel argument applies to allergen sensitization and, similarly, predicts greater muscle shorten-

ing (Fig 2). Allergen sensitization is known to cause increases in the shortening velocity and bridge-cycling rates; these changes are thought to be attributable to increased expression of myosin light-chain kinase.^{72,74,76} The extent to which increases in the bridge-cycling rate caused by allergen sensitization translate into excessive airway narrowing is now being explored; potential synergistic effects of allergy in combination with the maturing lung are unknown. Nonetheless, the theory of perturbed myosin binding leads us, at long last, to the first plausible mechanism by which the rate of bridge cycling and its regulation may be reasonably thought to bear on the prevalence of childhood asthma and its changes with lung maturation and allergic status (Figs 2 and 3).

UNNATURAL ACTS

In reexamining our understanding of airway hyperresponsiveness from the point of view of perturbed myosin binding, it becomes clear in retrospect that we might have misinterpreted some central observations in the past. At the level of isolated muscle, for example, isometric contraction and unloaded shortening have taught us a lot about the underlying biophysics of muscle, but

these maneuvers are unnatural acts that are restricted mainly to the laboratory. They bear at most a rather indirect relationship to how the muscle will behave during physiologic loading. Compounding this problem, the laboratory of Pratusевич et al⁵³ has shown that the optimal muscle length, a core concept in skeletal muscle, may not exist in airway smooth muscle but rather may be an artifact of how we have chosen to characterize muscle in the laboratory. We now know that airway smooth muscle can rapidly adapt to generate the same maximum force over a wide range of muscle lengths.

At the level of the intact patient, spirometry has been the method used most often to characterize airway responsiveness to bronchial provocation, with key indices of airway obstruction and airway responsiveness to inhaled agonists being derived from changes in lung volume in time over the course of a forced deflation after a deep inspiration to total lung capacity. The simple notion that we use all the time in spirometry is that the flow that can be achieved at any volume is a rough index of airway size and therefore a measure of the state of airway narrowing. However, spirometry too is an unnatural act, and one that takes Heisenberg's uncertainty principle to an absurd limit. Except in the case of asthmatic subjects with spontaneous airways obstruction (who have lost the ability to dilate their airways with a deep inspiration), the test virtually ablates the very contractile response that it purports to measure. This is not necessarily such a bad thing, however. The implication is that airway responsiveness measured by using standard spirometry does not measure the airway narrowing that results from exposure to non-specific agonists as much as it measures the extent to which a deep inspiration relaxes airway smooth muscle or fails to do so. If true, then standard spirometry during bronchial provocation testing is not so much an assay of the acute airway narrowing that occurs in response to challenge but rather is an assay of the dilator response that occurs in response to a deep inspiration.

IT'S ABOUT TIME

The appeal of perturbed myosin binding is how simple it makes everything else, but the implications ought not to be pressed too far because these perturbed states remain largely uncharacterized biochemically, metabolically, and structurally. Nonetheless, the unifying idea is that the caliber of the airway lumen during muscle activation is set by a nonequilibrium process and not a balance of static forces. With each breath force, fluctuations impinge on the muscle, and the myosin binding is perturbed anew, decreasing bridge numbers, increasing their rate of turnover, and maintaining the system far away from equilibrium conditions, although only at a certain energetic expense. The hypothesis suggests that destabilization of this dynamic and the resulting collapse to a static binding equilibrium may be the primary mechanical consequence of the cytokine-driven thickening of the peribronchial adventitia that is associated with inflammatory remodeling of the airway in asthma. In this case force fluctuations

acting on the muscle would be buffered, and the muscle would then shorten, stiffen, and find itself stuck in that state for as long as the muscle remains activated (Fig 2). If so, this frozen state would at long last provide a mechanism to explain the impairment in the ability of lung inflations to dilate the airway in asthma.

Of course, this picture may well be incorrect, and it is almost certainly incomplete. In the context of the airway in equilibrium circumstances, Macklem⁷⁷ has pointed out that once the muscle has become maximally activated (and, we would now say, come to equilibrium) it is the forces and the loads that become all important and that the level of the plateau response becomes essentially uncoupled from underlying biochemistry and cell biology. The static balance of which Macklem spoke remains highly relevant because it is the static state to which the dynamically equilibrated system would collapse if destabilized, but this picture is now seen to be incomplete. The view of the airway as a static mechanical system has given way to the more general idea of the airway as a system that is intrinsically dynamic, conditionally stable, and far away from static mechanical equilibrium. If this new picture is correct, then normal degrees of airway narrowing (normoreactivity) would have to be viewed in the context of a dynamic system that is tightly integrated across scales, with actomyosin reaction kinetics and cytoskeletal plasticity within the airway smooth muscle coupled directly to organ-level events like tidal fluctuations in transpulmonary pressure. Accordingly, the system would be controlled by kinetic parameters, which depend on rate processes and time, bridge-cycling rates, the frequency of breathing, and the time between sighs, and not just static parameters, where rates and time can never be factors. The pathobiology (ie, collapse to static equilibrium conditions and excessive airway narrowing) would then be seen to be a consequence of the failure of that coupling. In that case the muscle would shorten to a length dictated by static equilibrium conditions and remain frozen there largely independent of respiratory events transpiring at the organ level (Fig 2).

MAY THE FORCE BE WITH YOU

Recent advances in murine models have deepened our understanding of the immunologic and genetic bases of airway hyperresponsiveness and have shown that airway hyperresponsiveness can be uncoupled from airway inflammation,^{70,71} but we understand little of the specific mechanistic processes by which these factors lead to airway hyperresponsiveness and bronchospasm. The integrated point of view described in this review reinforces the notion that the inflammatory processes associated with asthma affect more than the pharmacology of contractile agonists and the signal transduction cascade within the contractile apparatus. Rather, muscle activation is but one facet of a multifaceted inflammatory process that also causes cytokine-driven remodeling of airway connective tissues, alteration of airway smooth muscle mass, and modifications of the processes that reg-

ulate cross bridge-cycling rates. These very factors may conspire to destabilize a conditionally stable dynamic system that is far from equilibrium but is always flirting with disaster, like a spinning top that is wobbling but has not yet tumbled to rest. Should these factors change in concert, as they undoubtedly do in inflammatory airway disease, this might explain how rather small changes in each that, if taken alone, might seem inconsequential but when taken collectively might be sufficient to destabilize the process and, in doing so, precipitate a collapse to static conditions and a disproportionately large decrement of airway function.

The perturbed equilibrium hypothesis is attractive because it is rather simple yet seems to stitch together within a unified framework, a diverse group of respiratory disease phenotypes that were largely unexplained and had been thought to be essentially unrelated (Fig 3). This integrated point of view suggests that as each of us focuses narrowly on investigation of a particular mechanism to explain airway obstruction and airway hyperresponsiveness in asthma, we must bear in mind that all of these mechanisms may well contribute. In a system that is conditionally stable and perched precariously at the brink of destabilization, there may be no single cause responsible for its collapse, just as the last straw that breaks the camel's back is neither more nor less the cause than the first. Dynamic equilibration of the airway lumen during bronchial provocation may be an emergent phenomenon that is sustained only by a variety of interconnected factors comprising a web of causality, and every contributing factor may be important. Each is a bit like the brush stroke in an impressionistic painting: too close and all you see is blotches, but farther away, the image snaps into focus. One of the major challenges for the asthma community over the next several years will be to establish which factors comprise the essential determinants of airway narrowing and how they fit together.

I thank Peter Macklem, Solbert Permutt, and Roland Ingram, Jr, whose thinking on this subject has very much influenced my own.

REFERENCES

1. Brown RH, Mitzner W. The myth of maximal airway responsiveness in vivo. *J Appl Physiol* 1998;85:2012-7.
2. Warner DO, Gunst SJ. Limitation of maximal bronchoconstriction in living dogs. *Am Rev Respir Dis* 1992;145:553-60.
3. Ding DJ, Martin JG, Macklem PT. Effects of lung volume on maximal methacholine-induced bronchoconstriction in normal humans. *J Appl Physiol* 1987;62:1324-30.
4. Gunst SJ, Stropp JQ, Service J. Mechanical modulation of pressure-volume characteristics of contracted canine airways in vitro. *J Appl Physiol* 1990;68:2223-9.
5. Woolcock A, Salome CM, Yan K. The shape of the dose-response curve to histamine in asthmatic and normal subjects. *Am Rev Respir Dis* 1984;130:71-5.
6. Moreno R, Hogg JC, Paré PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986;133:1171-80.
7. Macklem PT. A theoretical analysis of the effect of airway smooth muscle load on airway narrowing. *Am J Respir Crit Care Med* 1996;153:83-9.
8. Gunst SJ, Warner DO, Wilson TA, Hyatt RE. Parenchymal interdependence and airway response to methacholine in excised dog lobes. *J Appl Physiol* 1988;65:2490-7.
9. Lambert RK, Paré PD. Lung parenchymal shear modulus, airway wall remodeling, and bronchial hyperresponsiveness. *J Appl Physiol* 1997;83:140-7.
10. Thomson RJ, Bramley AM, Schellenberg RR. Airway muscle stereology: implications for increased shortening in asthma. *Am J Respir Crit Care Med* 1996;154:749-57.
11. Wiggs BR, Bosken C, Paré PD, James A, Hogg JC. A model of airway narrowing in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;145:1251-8.
12. Black J. Role of airway smooth muscle. *Am J Respir Crit Care Med* 1996;153:S2-4.
13. Stephens NL, Laviolette M, Unruh H, Ma X. Contractility in bronchial airway smooth muscle cells obtained from asthmatic subjects by endobronchial biopsy is increased [abstract]. *Am J Respir Crit Care Med* 1998;157:A746.
14. Murphy TM, Mitchell RW, Halayko A, et al. Effect of maturational changes in myosin content and morphometry on airway smooth muscle contraction. *Am J Physiol* 1991;260:L471-80.
15. Nagase T, Fukuchi Y, Dallaire MJ, Martin JG, Ludwig MS. In vitro airway and tissue response to antigen in sensitized rats: role of serotonin and leukotriene D4. *Am J Respir Crit Care Med* 1995;152:81-6.
16. Mead J, Takashima T, Leith D. Stress distribution in lungs: a model of pulmonary elasticity. *J Appl Physiol* 1970;28:596-607.
17. Moreno RH, Lisboa C, Hogg JC, Paré PD. Limitation of airway smooth muscle shortening by cartilage stiffness and lung elastic recoil in rabbits. *J Appl Physiol* 1993;75:738-44.
18. Kallok MJ, Lai-Fook SJ, Hajji MA, Wilson TA. Axial distortion of airways in the lung. *J Appl Physiol* 1983;54:185-90.
19. Lai-Fook SJ. Elasticity analysis of lung deformation problems. *Ann Biomed Eng* 1981;9:451-62.
20. Paré PD, Wiggs BR, James A, Hogg JC. The comparative mechanics and morphology of airways in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991;143:1189-93.
21. Jeffery PK. Investigation and assessment of airway and lung inflammation: we now have the tools, what are the questions? *Eur Respir J* 1998;11:524-8.
22. Holgate ST, Lackie PM, Davies DE, Roche WR, Walls AF. The bronchial epithelium as a key regulator of airway inflammation and remodeling in asthma. *Clin Exp Allergy* 1999;29(Suppl 2):90-5.
23. Hai CM, Murphy RA. CA2+, crossbridge, phosphorylation, and contraction. *Annu Rev Physiol* 1989;51:285-98.
24. Dillon PF, Aksoy MO, Oriska SP, Murphy RA. Myosin phosphorylation and the crossbridge cycle in arterial smooth muscle. *Science* 1987;211:495-7.
25. Butler TM, Siegman MJ. Control of cross-bridge cycling by myosin light chain phosphorylation in mammalian smooth muscle. *Acta Physiol Scand* 1998;164:389-400.
26. Haeberle JR, Hemric ME. A model for the coregulation of smooth muscle actomyosin by caldesmon, calponin, tropomyosin, and the myosin regulatory light chain. *Can J Physiol Pharmacol* 1994;72:1400-9.
27. Jones KA, Lorenz RR, Prakash YS, Sieck GC, Warner DO. ATP hydrolysis during contraction of permeabilized airway smooth muscle. *Am J Physiol* 1999;277:L334-42.
28. Horowitz A, Menice CB, Laporte R, Morgan KG. Mechanisms of smooth muscle contraction. *Physiol Rev* 1996;76:967-1003.
29. Sanders LC, Matsumura F, Bokoch GM, de Lanerolle P. Inhibition of myosin light chain kinase by p21-activated kinase. *Science* 1999;283:2083-5.
30. Kimura K, Ito M, Amano M, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 1996;273:245-8.
31. Weber LP, Van Lierop JE, Walsh MP. Ca2+-independent phosphorylation of myosin in rat caudal artery and chicken gizzard myofilaments. *J Physiol (Lond)* 1999;516:805-24.
32. Fredberg JJ, Inouye DS, Mijailovich SM, Butler JP. Perturbed equilibrium of myosin binding in airway smooth muscle and its implications in bronchospasm. *Am J Respir Crit Care Med* 1999;159:1-9.
33. Fish JE, Ankin MG, Kelly JF, Peterman VI. Regulation of bronchomotor tone by lung inflation in asthmatic and nonasthmatic subjects. *J Appl Physiol Respir Environ Exerc Physiol* 1981;50:1079-86.
34. Green M, Mead J. Time dependence of flow-volume curves. *J Appl Physiol* 1974;37:793-7.
35. Skloot G, Permutt S, Togias A. Airway hyperresponsiveness in asthma: a

- problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 1995;96:2393-403.
36. Orehek J, Charpin D, Velardocchio JM, Grimaud C. Bronchomotor effect of bronchoconstriction-induced deep inspirations in asthmatics. *Am Rev Respir Dis* 1980;121:297-305.
 37. Wheatley JR, Pare PD, Engel LA. Reversibility of induced bronchoconstriction by deep inspiration in asthmatic and normal subjects. *Eur Respir J* 1989;2:331-9.
 38. Brusasco V, Crimi E, Barisione G, Spanevello A, Rodarte JR, Pellegrino R. Airway responsiveness to methacholine: effects of deep inhalations and airway inflammation. *J Appl Physiol* 1999;87:567-73.
 39. Shen X, Gunst SJ, Tepper RS. Effect of tidal volume and frequency on airway responsiveness in mechanically ventilated rabbits. *J Appl Physiol* 1997;83:1202-8.
 40. Nadel JA, Tierney DF. Effect of a previous deep inspiration on airway resistance in man. *J Appl Physiol* 1961;16:717-9.
 41. Bendixen HH, Smith GM, Mead J. Pattern of ventilation in young adults. *J Appl Physiol* 1964;19:195-8.
 42. Fredberg JJ, Inouye D, Miller B, et al. Airway smooth muscle, tidal stretches, and dynamically determined contractile states. *Am J Respir Crit Care Med* 1997;156:1752-9.
 43. Lim TK, Pride NB, Ingram RH Jr. Effects of volume history during spontaneous and acutely induced air-flow obstruction in asthma. *Am Rev Respir Dis* 1987;135:591-6.
 44. Lim TK, Ang SM, Rossing TH, Ingenito EP, Ingram RH Jr. The effects of deep inhalation on maximal expiratory flow during intensive treatment of spontaneous asthmatic episodes. *Am Rev Respir Dis* 1989;140:340-3.
 45. Drazen JM, Austen KF. Leukotrienes and airway responses. *Am Rev Respir Dis* 1987;136:985-98.
 46. Ingram RH Jr. Relationship among airway-parenchymal interactions, lung responsiveness, and inflammation in asthma. *Chest* 1995;107:148S-52S.
 47. King GG, Moore BJ, Seow CY, Pare PD. Time course of increased airway narrowing caused by inhibition of deep inspiration during methacholine challenge. *Am J Respir Crit Care Med* 1999;160:454-7.
 48. Moore BJ, Verburg LM, King GG, Pare PD. Effect of deep inspiration on methacholine dose-response curves in normal subjects. *Am J Respir Crit Care Med* 1997;156:1278-81.
 49. Mijailovich M, Butler J, Fredberg J. Perturbed equilibria of myosin binding in airway smooth muscle: bond-length distributions, mechanics and ATP metabolism. *Biophys J*. In press.
 50. Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airway dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1993;148:1220-5.
 51. Macklem PT. A hypothesis linking bronchial hyperreactivity and airway inflammation: implications for therapy. *Ann Allergy* 1990;64:113-6.
 52. Gunst SJ, Meiss RA, Wu M-F, Rowe M. Mechanisms for the mechanical plasticity of tracheal smooth muscle. *Am J Physiol* 1995;268:C1267-76.
 53. Pratushevich VR, Seow CY, Ford LE. Plasticity in canine airway smooth muscle. *J Gen Physiol* 1995;105:73-94.
 54. Nocolis G, Prigogine I. Exploring complexity. New York: WH Freeman; 1989.
 55. Nocolis G, Prigogine I. Exploring complexity. New York: Freeman; 1989.
 56. deGennes P. Scaling concepts in polymer physics. Ithaca (NY): Cornell University Press; 1979.
 57. Fredberg JJ, Jones KA, Nathan M, et al. Friction in airway smooth muscle: mechanism, latch and implications in asthma. *J Appl Physiol* 1996;81:2703-12.
 58. Froeb HF, Mead J. Relative hysteresis of the dead space and lung in vivo. *J Appl Physiol* 1968;25:244-8.
 59. Lakser O, Fredberg JJ. The p38 MAP kinase inhibitor SB203580 increases the effect of load fluctuations on airway smooth muscle length [abstract]. *Am J Respir Crit Care Med* 2000;161:A281.
 60. Hedges JC, Dechert MA, Yamboliev IA, et al. A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 1999;274:24211-9.
 61. Tepper R, Shen X, Bakan E, Gunst SJ. Maximal airway responses in mature and immature rabbits during tidal ventilation. *J Appl Physiol* 1995;79:1190-8.
 62. Pare PD, Roberts CR, Bai TR, Wiggs BJ. The functional consequences of airway remodeling in asthma. *Monaldi Arch Chest Dis* 1997;52:589-96.
 63. Eden E, Mitchell D, Mehlman B, et al. Atopy, asthma and emphysema in patients with severe alpha-1-antitrypsin deficiency. *Am J Respir Crit Care Med* 1997;156:68-74.
 64. Singas E, Lesser M, Spungen A, Bauman WA, Almenoff PL. Airway hyperresponsiveness to methacholine in subjects with spinal cord injury. *Chest* 1996;110:911-5.
 65. Sparrow D, O'Connor GT, Rosner B, Weiss ST. Predictors of longitudinal change in methacholine airway responsiveness among middle-aged and older men: the Normative Aging Study. *Am J Respir Crit Care Med* 1994;149:376-81.
 66. Boyer J, Amin N, Taddonio R, Dozor AJ. Evidence of airway obstruction in children with idiopathic scoliosis. *Chest* 1996;109:1532-5.
 67. Camargo CA Jr, Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch Intern Med* 1999;159:2582-8.
 68. Martin RJ, Pak J, Irvin CG. Effect of lung volume maintenance during sleep in nocturnal asthma. *J Appl Physiol* 1993;75:1467-70.
 69. Irvin CG, Pak J, Martin RJ. Airway-parenchyma uncoupling in nocturnal asthma. *Am J Respir Crit Care Med* 2000;161:50-6.
 70. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;157:4-9.
 71. Haley KJ, Drazen JM. Inflammation and airway function in asthma—what you see is not necessarily what you get. *Am J Respir Crit Care Med* 1998;157:1-3.
 72. Fan T, Yang M, Halayko A, Mohapatra SS, Stephens NL. Airway responsiveness in two inbred strains of mouse disparate in IgE and IL-4 production. *Am J Respir Cell Mol Biol* 1997;17:156-63.
 73. Chitano P, Wang J, Cox CM, Stephens NL, Murphy TM. Different ontogeny of rate of force generation and shortening velocity in guinea pig trachealis. *J Appl Physiol* 2000;88:1338-45.
 74. Jiang H, Rao K, Halayko AJ, Liu X, Stephens NL. Ragweed sensitization-induced increase of myosin light chain kinase content in canine airway smooth muscle. *Am J Respir Cell Mol Biol* 1992;7:567-73.
 75. Low RB, Mitchell J, Woodcock-Mitchell J, Rovner AS, White SL. Smooth-muscle myosin heavy-chain SM-B isoform expression in developing and adult rat lung. *Am J Respir Cell Mol Biol* 1999;20:651-7.
 76. Jiang H, Rao K, Liu X, Liu G, Stephens N. Increased Ca²⁺ and myosin phosphorylation, but not calmodulin activity in sensitized airway smooth muscles. *Am J Physiol* 1995;268:L739-46.
 77. Macklem PT. The clinical relevance of respiratory muscle research. *Am Rev Respir Dis* 1986;134:812-5.