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The smooth muscle and airway hyperresponsiveness

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Abstract. – **Airway hyperresponsiveness, excessive airway narrowing caused by stimuli that normally elicit limited or no response, is one of the cardinal features of asthma.**

The length-dependence of smooth muscle contractility has been recognized for decades, and it forms an essential foundation for many aspects of the physiological regulation of airway contractility in vivo.

This review summarizes the structural and functional alterations of airway smooth muscle in asthma and chronic obstructive pulmonary disease, that underlie pathophysiological conditions of airway hyperresponsiveness.

Key Words:

Smooth muscle, Bronchial hyperresponsiveness, Asthma, COPD.

Introduction

Airway smooth muscle was first described in detail in 1822 by Reisseisen[1]. This and other descriptive studies[2-5] showed that airway smooth muscle is present in the central and peripheral airways, relatively more prominent in the peripheral airways, more transverse in central airways and slightly more longitudinal in peripheral airways, and arranged in a helical or geodesic pattern which is more apparent in peripheral airways and when the lung is fully inflated.

Numerous studies[2,6-10] have shown that the airway smooth muscle occupies relatively more of the airway wall in the peripheral compared with the central airways. In absolute terms, the amount of airway smooth muscle is lesser in the peripheral airways. A number of studies[8,9,11-13] have standardized the amount of smooth muscle in the airway wall by measuring the total cross-sectional area of smooth muscle seen on a transverse section of the airway and dividing it by the length of the basement membrane (the perimeter of the airway) since this is independent of the degree of airway smooth muscle contraction and lung inflation[14,15]. The airway smooth muscle encircles the airway completely below the level of the trachea. General descriptions of airway smooth muscle[5] have suggested that its orientation is transverse in the trachea and that it forms a helical or geodesic pattern around the central and peripheral airways. The angle of orientation may depend upon the degree of inflation of the lung, being more openmeshed at full inflation[5], which tends to maximize the angle of orientation. However, there are few quantitative studies.

Although the role of smooth muscle in the airway remains in the realm of conjecture, it certainly causes variation in airway calibre and airway wall compliance, and contraction of airway smooth muscle is almost certainly necessary for the excessive airway narrowing that is observed in asthma. Its importance in other diseases such as chronic obstructive pulmonary disease and chronic bronchitis is probably less since airflow obstruction is fixed and administration of inhaled bronchoconstrictors results in only limited smooth muscle shortening, unlike that seen in asthma. In asthma, the sudden and intermittent nature of airway narrowing and the response of the symptoms to

smooth muscle agonists and antagonists have given rise to the suggestion that abnormalities of airway smooth muscle may be the primary abnormality. The mechanisms involved are not clear.

The first systematic study of the amount of airway smooth muscle in asthmatic subjects was conducted by Huber and Koessler[16]. These investigators recognized that airway wall and smooth muscle layer thickness would appear to be increased in asthmatic subjects if the lumenal diameter were to be used as a marker of the airway size. This is because the muscle would be shortened and thickened and the lumen would be narrowed. The theories that have been formulated to explain the attacks of paroxysmal dyspnea are agreed today that the difficulty of respiration is due to a stenosis of the bronchi, but whether this narrowing is due chiefly or exclusively to a spasm of the bronchial smooth muscle system, to swelling and exudation of the bronchial mucosa, to a true obturation stenosis by the secretion from the bronchial glands, or to a combination of two or more of these conditions seems still to be an unsettled question[16].

From the standpoint of smooth muscle alterations , excessive airway narrowing could be brought about by: (1) an increased amount of smooth muscle with normal contractile properties; (2) altered smooth muscle cellular characteristics allowing for increased contractility of individual cells; (3) less compliant intercellular components connecting myocytes; and (4) decreased stiffness of the parallel elastic elements, either within myocytes or within connective tissue elements between and surrounding myocytes.

The single most important broad-brush fact about any muscle is that it contracts when activated, and so it is with airway smooth muscle. Airway smooth muscle may be distinct from muscles in other organ systems, however, in that muscle contraction is usually thought of as being a normal process occurring within a physiological system that either creates motion (in the case of skeletal and cardiac muscle systems) or sets tone or stabilizes shape (in the case of hollow organ systems). In the case of airway smooth muscle, no consensus has emerged as to what useful function contraction might play. There is no known disease entity or appreciable physiological deficit that is associated with loss of airway smooth muscle contractility. Rather, it seems that, when it is operating normally, airway smooth muscle may have no compelling function and, when it contracts excessively or contracts even moderately within an altered microenvironment, serves only to cause problems.

Functional properties airway smooth muscle were considered by Fay[17] and Dixon and Brodie[18]. More recent studies have shown that the fraction of the tissue volume that is attributable to contractile machinery is comparable for airways, alveolated ducts, and blood vessels in the lung parenchyma[19]; the lung parenchyma, like the airway, is a contractile tissue[20-24].

Identification of the normal physiological role of airway smooth muscle remains elusive; in that regard, airway smooth muscle stands in contrast with other smooth muscle systems whose primary functional roles are self-evident. Mead[25] questioned the extent to which changes of smooth muscle tone might play some homeostatic role to stabilize airways and airspaces. He speculated that a moderately constricted state of airway smooth muscle may make airways behave more like the lung parenchyma in which they are embedded, thus improving the homogeneity of lung expansion; he reasoned that homogeneous lung expansion might depend on mechanical interdependence among lung structures all operating on a background of smooth muscle activity. It has been argued by others that contraction of airway smooth muscle might serve to modulate the tradeoff between dead space vs. airway resistance in a way that minimizes the work of breathing[26], serve to adjust airway caliber among parallel pathways and parenchymal compliance among peripheral lung regions in a way that optimizes the distribution of ventilation[19,27], serve to narrow the airway in a way that improves the ability of cough to expel worms or other foreign objects from the airway, serve to stiffen the airway sufficiently to prevent extreme airway collapse during forced expiration[28], or serve to match the mechanical hysteresis of small airways and alveolated ducts to the rather appreciable mechanical hysteresis of the alveolar surface film in a way that allows for synchronous and uniform alveolar expansion[25,29,30].

Methods

Mechanical Factors Influencing Airway Narrowing

The airway wall in asthma and chronic obstructive pulmonary disease is markedly thickened. It has also been observed that when the smooth muscle constricts the mucosa buckles, forming folds that penetrate into the airway lumen. This folding pattern may influence the amount of luminal obstruction associated with smooth muscle activation.

Mechanical factors are of great importance in determining the magnitude of bronchoconstriction induced by contractile agonists. Although support for this notion rests on circumstantial evidence rather than direct proof, bronchoconstriction is a function of airway smooth muscle shortening, and thus of the mechanical forces acting to limit such shortening. It seems very likely that airway responsiveness reflects the balance between forces contracting airway smooth muscle and the impedance to shortening of that muscle. Excised airway smooth muscle has an exceptional capacity to shorten in vitro (by 80 ± 90% of its initial length) when stimulated. Shortening of this degree would result in complete airway closure if it were to take place in vivo. As airway closure does not appear to occur to any great extent following supramaximal stimulation in normal subjects, potent factors must exist that limit the degree of airway narrowing in vivo[31].

The observation that breathing at low lung volumes greatly enhances the magnitude of airway narrowing whereas high volumes are "bronchoprotective"[32] has been interpreted as evidence that the parenchymal attachments to the airway wall impose an impedance to airway narrowing. This impedance results from the elastic recoil of the parenchyma as well as from the forces of interdependence between the airway wall and the parenchymal attachments. Simply stated, the elastic recoil of the lung parenchyma helps to keep the airways open by transmitting a pressure equivalent to pleural pressure through the parenchymal attachments to the outer aspect of the airway walls. Indeed, the protective effect of these forces is amplified as a function of the degree of bronchoconstriction[33]. The airway wall itself is likely to resist compression by causing contraction of airway smooth muscle. The constitutive properties of the airway wall that help to prevent airway closure are likely to vary as a function of airway generation; airway cartilage is one of the components that resists airway compression. It has been postulated that alterations in the cartilage in asthma, through the action of proteolytic enzymes released by infiltrating leukocytes, might allow the airways to narrow more easily[34]. The mucosal surface of the airway wall forms folds which may vary in number as a function of both airway wall dimensions and wall material properties[35].

The formation of mucosal folds may also lead to an alteration in surface forces in the region of these interstices, causing a reduction in pressures and favouring transudation of fluid and further retraction of the airways[36].

The magnitude of the obstructive response observed in asthma for a given contractile stimulus reflects the capacity of airway smooth muscle and the load against which it acts, i.e., the structural components of the parenchyma and airway wall. Despite its critical role in this process, little is known about airway wall mechanics, especially in chronic asthma in which there is significant remodeling of the wall[11,37].

This remodeling no doubt contributes to the distinctive change in the pattern of buckling observed in airways, which is now believed to contribute to the asthmatic response.

The thickening of the epithelial basal lamina has been considered a dominant feature of asthma[38]. Jeffery et al.[39] have made detailed measurements of the subepithelial collagen layer and have noted a markedly thickened reticular lamina. Roberts[40] commented that this distinct layer is doubled in thickness from ~7 to 14 µm in asthmatic subjects relative to nonasthmatic controls and that this thickened layer is composed of mainly types III and V collagen. It has been suggested that this newly deposited collagen derives from myofibroblasts that populate this layer in asthmatics[38]. Roche et al.[37] have commented that the collagen fibers in this subepithelial region appear more densely packed than normal, and Roberts[40] has hypothesized that this would lead to a more mechanically stiff layer relative to the surrounding extracellular matrix.

Increased airway narrowing in response to nonspecific stimuli is a characteristic feature of human obstructive airways diseases. This abnormality is an important aspect of the disease, although the pathophysiological changes leading to this hyperresponsiveness are still unknown. Several mechanisms have been postulated to explain hyperresponsiveness, including alterations in the neurohumoral control of airway smooth muscle[41,42], increased sensitivity of airway smooth muscle[43], increased mucosal permeability[44], increased mucosal secretions[45,46], and mechanical factors related to remodeling of the airways[9,47]. In spite of the potential contributions of some of these mechanisms, the reversibility and rapid onset of airway narrowing indicate that airway smooth muscle contraction is central to the pathophysiology underlying exaggerated airway narrowing.

The mechanism that prevents noncartilaginous airways from collapsing due to airway smooth muscle contraction is still not completely understood. It has been suggested that mechanical loading from lung parenchyma and within the airway wall may attenuate the shortening of the smooth muscle in normal subjects and that a decrease in the loads may explain the exaggerated airway narrowing observed in diseases such as asthma.

Airway smooth muscle in vitro can shorten to < 25% of its resting length under zero load[48]. With this amount of shortening, airway smooth muscle would certainly close all airways in the lung if there is no resisting force to counteract the force generated by the muscle[49]. There are at least two sources of resistive force that have been considered. One is the tethering of the airway smooth muscle wall to lung parenchyma[50]; the elastic parenchyma provides a radial force through the tethers of alveolar attachments and counteracts the force generated by the constricting muscle. Another possible source of resistive force is from the folding of basement membrane[35,46,51].

The elastic load provided by the parenchyma was found to be insufficient to explain the observed limitation of shortening of the airway smooth muscle[14,50,52].

Estimation of the resistive force provided by the folding basement membrane depends on knowledge of flexural rigidity of the membrane, which is still an unknown.

Experimental data and mathematical models suggest that alterations in the mass of airway smooth muscle or changes in its contractile properties may explain the excessive airway narrowing in asthma. However, the interaction between the contracting smooth muscle and the surrounding structures, including the constituents of the airway wall and lung parenchyma, may be of equal importance. In particular, the cyclical stresses applied to airway smooth muscle by tidal breathing or episodic deep breaths may serve an important function in maintaining the airways in a dilated state. Several biochemical changes of potential importance to airway hyperresponsiveness, including increased activity of myosin light chain kinase and phospholipase C, have been shown to result from allergic sensitization. Perhaps alterations in the dynamic properties of airway smooth muscle, such as increases in the velocity of shortening, may permit the muscle to better resist the dilating influence of cyclical stress imposed by tidal breathing. Whether hyperplastic airway smooth muscle undergoes changes in its dynamic properties merits exploration[53].

Airway Smooth Muscle Remodelling in Asthma

Patients with long-standing and severe asthma often develop poorly reversible airway obstruction that is refracory to bronchodilatory and anti-inflammatory medication[54,55]. This complication is associated with the development of persistent structural changes in the airway wall[56,57]. These include remodelling of tissue elements in the basement membrane and in the airway tissue in general. Of these, the most striking is an increase in mural smooth muscle content.

Although other components of airway wall remodelling may also be important in determining airways hyperresponsiveness (e.g., increased mucosal thickness and increased adventitial thickening) some studies[58,59], forms the basis of the current belief that the major smooth muscle abnormality that contributes to the development of airway wall thickening in chronic severe asthma involves excessive muscle hyperplasia and hypertrophy. Accordingly, investigators are now addressing the cellular and molecular mechanisms which drive the increase in muscle in this tissue, although, to an extent, this has been at the ex-

pense of investigating other aberrant responses of smooth muscle such as increased cell survival or deposition of extracellular matrix proteins within the myo-bundles[60]. Conceivably, each of these processes may operate to varying degrees in chronic severe asthma, and further studies are required to determine their relative importance to the real or apparent increase in airway wall smooth muscle content that is observed in vivo.

Although asthma typically involves reversible airflow obstruction, in some asthmatics this obstruction is less reversible. In such patients, the obstruction is associated with persistent and largely irreversible structural changes in the airway wall, an important component of which is the increased mural smooth muscle content that arises due to repeated and intense stimulation of the smooth muscle by contractile agonists, pro-inflammatory mediators and polypeptide growth factors. Recently, it has been hypothesized that the contribution that this increase in muscle mass makes to the pathogenesis of chronic asthma may not be limited to simple geometric obstruction (by increased airway wall thickening), but may also involve reversible phenotypic modulation of the muscle from a contractile to a more synthetic/proliferative state in which additional functions such as the production and release of pro-inflammatory mediators, growth factors and extracellular matrix elements are more apparent. It is now known that airway wall smooth muscle is not composed of a homogeneous population of cells, and that altered cellular heterogeneity may be an important factor in the pathogenesis and progression of asthma. It is also known from in vitro studies that, although many mediators can increase smooth muscle proliferation, similarities are present across species in the intracellular mechanisms activated by these mediators. The potential effects, however, of smooth muscle phenotypic modulation on intracellular signalling events are unknown. For the moment, it appears that diverse extracellular stimuli can induce cell growth by activation of common intracellular signalling pathways.

Control of Contractile Apparatus

The airway smooth muscle cell is critically important in asthma, mediating not only the bronchoconstrictor effects of agents such as histamine and methacholine but also the bronchodilator effects of β_2-agonists. In principle, an understanding of the mechanisms underlying control of the airway smooth muscle cell is important in defining the pathophysiological abnormalities present in asthma and other airway diseases. Both the contractile and relaxant responses of airway smooth muscle are regulated by cross-talk between the important intracellular signalling pathways controlling contraction and relaxation. Although many of these pathways are complex, it is important that understanding of these processes is increased. Most therapeutic agents currently in use as bronchodilators interact with these pathways, and novel targets exist which can potentially be exploited in the development of new asthma therapies. Following stimulation of airway smooth muscle by classical contractile agonists such as histamine or methacholine, initiation of the contractile response depends upon stimulation of phospholipase C-dependent pathways[61,62].

These pathways have been extensively studied in cultured and noncultured airway myocytes from a range of species, although, because of difficulty in obtaining adequate amount of tissue, the majority of human studies have been performed using cultured cell systems[63].

Effect of Deep Inspiration

Asthma is characterized by airway lability and inflammation. The former phenomenon is probably related to that of bronchial hyperresponsiveness, defined as increased obstructive response to various chemical and physical stimuli[64]. Although hyperresponsiveness and airway inflammation are thought to be etiologically linked, the mechanism for such a link is elusive. It has become apparent to many investigators in this field that better understanding of the nature of airway hyperresponsiveness is warranted before the link to inflammation can be unveiled. In 1995, Skloot et al.[65] showed that, when a methacholine inhalation challenge is carried out under conditions where deep inspirations are prohibited, the response to increasing methacholine concentrations is similar in asthmatic and healthy subjects. In that study, they found that the airway responsiveness of nonasthmatic subjects, when assessed by partial (from end-

tidal inspiratory to residual volume) forced expiratory maneuvers, was not much different from that of asthmatics. Those observations indicated that lung inflation (through deep inspiration) had a potent beneficial effect on human airways and that this effect was markedly diminished or absent in asthma. Skloot et al.[65] proposed that lung inflation induced bronchodilation and speculated that, in asthma, the phenomenon of airway hyperresponsiveness to a direct spasmogen, such as methacholine, was merely a manifestation of the lack of the bronchodilatory ability of deep breaths. On the basis of the striking effect of deep inspirations on airways reponsiveness, it was expected that, after the induction of severe bronchoconstriction in the absence of deep inspirations, a sequence of deep breaths would rapidly return healthy airways to their baseline, nonconstricted state. The findings of that study disproved this hypothesis. Although the first deep breath taken after the end of the methacholine provocation produced a stronger bronchodilator response in the nonasthmatics, the overall ability of three deep inspirations to induce bronchodilation in this setting was not different between asthmatic and nonasthmatic subjects[65]. Given that other investigators[66] have documented the bronchodilatory ability of deep inspiration in experimental designs in which a full spirometric maneuver follows a partial maneuver, the explanation Skloot et al[65]. offered for their finding was that the potency of this bronchodilatory effect was significantly diminished in the face of preestablished significant smooth muscle contraction. This explanation, however, also raised some questions as to whether the striking difference between asthmatic and nonasthmatic subject, with respect to the beneficial effect of deep inspiration, could be solely attributed to a bronchodilator effect, which appeared quite susceptible to increased smooth muscle tone. This uncertainty led to the generation of another hypothesis, that deep inspirations not only have a bronchodilatory but also, most importantly, a bronchoprotective, effect on the airways.

The work of Skloot et al[65]. confirmed the previously reported effects of lung inflation in regulating bronchomotor tone[67-71]. Furthermore, Skloot et al.[65] demonstrated that the effect of deep inspiration was absent or diminished in asthma, offering support to a hypothesis that airway hyperresponsiveness in asthma may merely represent reduced ability of a deep inspiration to relax the airways. In the study of Skloot et al.[65], during the discussion of the potential mechanisms through which lung inflation benefits the airways, they referred to the work of Malmberg et al.[72] who, in an effort to develop an abbreviated methacholine bronchoprovocation for epidemiological studies, came across the observation that a deep inspiration before the administration of methacholine protected the airways from bronchoconstriction. In this work, it also appeared that the protective effect of deep inspiration was more pronounced than the effect obtained if deep inspiration took place after the administration of methacholine. A bronchoprotective effect of lung inflation implies that the increase in lung volume triggers a process that renders airway smooth muscle resistant to contractile stimuli. In contrast, the presence of a bronchodilating effect alone does not allow us to differentiate between a process that alters the state of airway smooth muscle and a mere stretch relaxation effect. Consequently, understanding the process behind lung inflation-induced bronchoprotection may lead to the elucidation of the mechanism of airway hyperresponsiveness in asthma, provided that healthy subjects and asthmatics are profoundly different in this respect. The significance of demonstrating that deep inspiration acts as a bronchoprotector lies in the fact that it can lead to new testable hypotheses regarding the mechanism through which lung inflation operates. The strong effect of deep inspiration, acting as a bronchoprotector in nonasthmatic subjects, explains the data of Burns and Gibson[73], who failed to show a significant response to methacholine in nonasthmatics using a modification of the protocol used by Skloot et al.[65]. The difference was that, in their protocol, each methacholine dose was inhaled with deep inspirations (as opposed to tidal inspirations) and was followed by measurement of partial flows 4 min later. Those deep inspirations conferred protection to the airways of the nonasthmatics and did not allow for bronchoconstriction to develop. Bronchoprotection against smooth muscle spasmogens is conferred by deep breaths. This function is dose dependent and

has remarkable potency. The bronchoprotective effect of deep inspiration is absent in asthma and may be a pivotal pathophysiologic abnormality in this disease[74].

Lung inflation has a stretching effect on the airways, especially the noncartilaginous airways. Although there is good reason to hypothesize that the stretch applied to the airways through a deep inspiration may account both for the ability to prevent and to reverse bronchial narrowing, the underlying mechanism through which airway stretching operates is still unknown, and the possibility of a different mechanism behind each of these two effects of deep inspiration also needs to be entertained. For example, stretching could cause a breakage in myosin-actin cross-bridges, leading to the relaxation of smooth muscle. This mechanism could explain the recovery from induced bronchoconstriction (i.e., the bronchodilatory capacity of deep inspirations); however, when a deep inspiration takes place under resting conditions, in which the number of myosin-actin bridges is relatively small and the rate of attachments is close to the rate of detachments induced by breathing[75], the additional disruption of myosin-actin interactions produced by a deep inspiration may not have enough of an effect on the airway smooth muscle to explain the dramatic phenomenon of bronchoprotection. Fredberg et al.[76] have recently advanced an interesting hypothesis. This hypothesis claims the presence of a dynamic equilibrium within airway smooth muscle that is modulated by the tidal stretches involved in normal breathing. A greater stretch imposed by deep inspiration in the resting state may put the muscle fibers into a condition of greater disequilibrium that makes the activation of smooth-muscle contraction more difficult. On the other hand, when contraction, and therefore equilibrium, has been reached, the energy required to disrupt this state is much higher. This is confirmed by *in vitro* observations by Gunst et al.[77] that volume oscillations applied to bronchi can prevent closure, and that once closure has been attained, large forces are required to open airways. Under this hypothesis, deep inspiration will be more effective before than after induced bronchoconstriction. An additional hypothesis regarding the mechanism through which stretch exerts its effects on the airways is that deep inspiration may induce changes in the status of airway smooth muscle through the release of relaxant factors. There is evidence, for example, that bronchodilating autacoids, such as prostaglandins E_2 and I_2, or neurotransmitters (e.g., vasoactive inhibitory polypeptide), are released in response to stretch[78]. More recently, a possible role in smooth-muscle relaxation has been suggested for endogenous nitric oxide (NO), as well as for atrial natriuretic peptide (ANP)[79,80]. In the heart, ANP is known to be released with stretching of atrial tissue; a similar mechanism may be operable in the lower airways. Another possibility is that stretch receptors in the airways are activated by deep inspiration, leading to central inhibition of parasympathetic tone. Unfortunately, there is little experimental evidence for or against the foregoing mechanisms, and they therefore remain purely speculative. The relative increase in the bronchodilatory and the bronchoprotective effects of deep inspiration in the context of an increased dose of a spasmogenic stimulusis worth discussing. One explanation for this increase may be that the forces of airways-parenchyma interdependence, which are thought to mediate lung inflation-induced airways stretching[32,81], may become stronger with increased bronchoconstriction, as demonstrated by Moreno et al[49]. This mechanism, however, would apply only to the bronchodilating and not to the bronchoprotective effects of deep inspiration; the latter are induced before the generation of smooth-muscle spasm by methacholine. Another explanation for the relative increase in bronchodilation and bronchoprotection with a spasmogenic stimulus is that the effect of deep inspiration is potent enough to overcome the smooth-muscle spasm induced even by higher doses of the spasmogen.

The limitation in the bronchodilatory capacity of deep inspiration is probably a consequence of the development of small airways obstruction and closure. Once muscle shortening has taken place, especially in the absence of stretch other than that from tidal oscillations, the muscle becomes resistant to the dilating effect of deep inspiration because it is approaching latch-state equilibrium[29,82]. In addition, with small airways closure, surface tension increases and large pressures are required to open the collapsed airways[83]. In

the absence of deep inspirations, healthy individuals develop bronchoconstriction with methacholine inhalation. One hypothesis is that deep inspiration results in bronchodilation. Deep inspirations did not protect the bronchi of asthmatics. Bronchoprotection is a potent physiologic function of lung inflation and established its absence, even in mild asthma. This observation deepens our understanding of airway dysfunction in asthma.

In previous studies, was showed that after approximately 10 min of activation of airway smooth muscle during which deep inspirations were inhibited, airway narrowing was increased and the bronchodilating effect of deep inspiration was impaired in normals[84]. If the bronchodilating effect of deep inspiration were impaired in asthmatics, then inhibition of deep inspirations during airway smooth muscle activation would not be associated with an increase in airway narrowing. Deep inspiration can affect acute airway narrowing by two mechanisms: "bronchoprotection" and "bronchodilation." Scichilone et al[85] studied bronchoprotection and bronchodilation in normal subjects and Wang and et al.[86] studied these mechanisms in an *in vivo* preparation of porcine airway smooth muscle. Bronchoprotection was defined in these studies as "the reduction in bronchoconstriction resulting from the act of deep inspiration before the airway smooth muscle has been stimulated to contract" whereas bronchodilation was the reduction in bronchoconstriction after airway smooth muscle activation by a spasmogen. Kapsali et al reported that the bronchoprotective effects of deep inspiration were stronger than the bronchodilatory effects and also that bronchoprotection was absent in asthmatics[87]. The effects of stretch on airway smooth muscle preparations were found to be consistent with the changes observed in *in vivo* studies[86], which suggests that the effects of deep inspiration on airway narrowing are indeed mediated by its action on airway smooth muscle.

It has been shown that in asthmatics there is impaired dilatation of the airways in response to stretch[65]. This is based on the differences in the response of asthmatic subjects to deep inspiration compared with normals. Fish et al[68] measured changes in airway conductance and partial flow-volume curves in response to methacholine inhalation in asthmatics and patients with allergic rhinitis before and after a deep inspiration. Although asthmatics showed greater airway narrowing as assessed by either measure, the difference between the asthmatics and rhinitics was much less prior to, than after deep inspiration. They suggested that failure of bronchodilatation with deep inspiration was an important cause of airways hyperresponsiveness in asthma. In asthmatics, the magnitude of the dilatation caused by deep inspiration varies with the degree of spontaneous airway narrowing; less dilatation is associated with a lower baseline FEV_1[88]. The response to deep inspiration may also vary depending on the cause of the airway narrowing. During a spontaneous exacerbation of asthma, the bronchodilating effect of deep inspiration is impaired and may even lead to further narrowing. After recovery when a similar degree of airway narrowing is reproduced by bronchial challenge, deep inspiration causes more dilatation than was observed during the asthma exacerbation[89]. More recently, Skloot et al performed standard and modified methacholine challenge tests in normals and asthmatics. During the standard challenge, spirometry and hence deep inspirations, were taken after each dose and during the modified challenge, deep inspirations were inhibited and only partial flow-volume curves were performed[65]. Using the time constant derived from the partial flow-volume curve as a measure of airway narrowing, they found that inhibition of deep inspirations during a methacholine challenge test in normals was associated with a degree of airway narrowing similar to that in asthmatics. However, inhibition of deep inspirations did not affect the dose-response curves in asthmatics. Furthermore, the findings were similar for FEV_1 measured at the end of the challenge tests, i.e., prohibition of deep inspiration augmented the decreases in FEV_1 in normals (approximately 10% versus 36%) but not in asthmatics (approximately 30% versus 32%). In another study in which isovolume flow on partial flow-volume curves at 25% of vital capacity (VC) was used as the measure of airway narrowing, the dose- response curves were also unchanged in asthmatics by deep inspiration inhibition but were shifted to the left in normals[90].

It has been known for many years that the response of asthmatic subjects to a deep inspiration differs from that observed in normal healthy subjects[91]. A deep inspiration causes a decrease in airway resistance in normal subjects, whereas asthmatics demonstrate either no change or a slight increase in airway resistance. Skloot et al[65] postulated that the inability to dilate airways during lung inflation is a primary defect in asthma. Their study[65] also showed that in the absence of a deep inspiration during methacholine (MCh) challenge, normal subjects had a greatly exaggerated and sustained response to this agonist. It was suggested that asthmatic airways could be modeled by this condition in normal subjects. Brusasco et al.[90], however, suggest that there are more intrinsic differences between the responses to lung inflation in airways from asthmatic and normal subjects. To better understand the mechanisms underlying this controversy, it is necessary to be able to assess the responses of airways directly, something that conventional pulmonary function tests in human subjects unfortunately cannot do. The size of the airways during normal tidal ventilation is also controlled by similar factors that determine the response to deep inspiration, i.e., the lung volume, the degree of smooth muscle activation, and the normal tidal stresses exerted by the parenchyma. The effect of such rhythmic cycling in decreasing the smooth muscle tone has been shown *in vitro*[92,93], and more recently, similar *in vivo* effects of tidal stresses have been documented[94]. Warner and Gunst[95] originally demonstrated that the rhythmic stretching associated with tidal breathing can decrease not only the baseline lung resistance, but also the response to MCh. This effect of tidal breathing limiting the degree of airway smooth muscle constriction was supported by the more recent studies of Tepper et al[94] and Shen et al[96]. This group proposed a mechanistic explanation that involves changes in the plasticity of the smooth muscle cellular cytostructure[97,98]. Another hypothesis that could account for the mechanism underlying these observations in humans was published by Fredberg et al[29,75]. It was proposed that the steady-state muscle force would be determined by a balance between high and low energy cross bridge dynamics. If the contractile stimulus is increased, then the number of cross bridges increases, and, given enough time at a constant load, the rapidly cycling cross bridges progressively convert to slowly cycling "latch" bridges, and the muscle stiffness increases. This model also predicted that the hysteresis of such latched smooth muscle would be decreased[29]. If this state occurred *in vivo*, then one might predict that there might be little effect of a deep inspiration in dilating the airways. Fredberg et al also speculated that normal tidal breathing provides a sufficient stress to keep the latch state from occurring in normal lungs[75]. A constrictor response to deep inspiration can be generated in normal airways by minimizing tidal stresses. The absence of these normal rhythmic stresses alters the smooth muscle throughout the airway tree, such that subsequent large stresses lead to a further constriction. This response may result from increased active smooth muscle tone after the deep inspiration. These results also offer a possible mechanism by which the response to deep inspiration is altered in asthmatic subjects[99].

Eosinophils, Leukotrienes and Bronchial Hyperresponsiveness

Among constitutively present cells, bronchoactive leukotrienes are produced predominantly by mast cells[100] and macrophages[101]. A unique characteristic of asthmatic inflammation is the migration of leukocytes from the peripheral blood to the conducting airways of the lung. This is especially true of the eosinophil, which is not present in the airways of normal individuals, but may be found in massive numbers during periods of airway hyperresponsiveness in asthmatic individuals[102,103]. Most experimental models indicate that eosinophils are an invariable component of asthmatic hyperresponsiveness, although some studies suggest that bronchoconstriction can occur in the relative absence of these cells. Leukotrienes have variable chemotactic properties that are highly cell dependent. There is also a strong species dependence which make data from other species unreliable for prediction of the human condition. The human eosinophil is weakly attracted by LTB_4, implying a weaker receptor population. By contrast, human neutrophils are strongly chemotactic and this was an initial concern in the development of anti-

leukotriene treatments directed against 5-lipoxygenase. The specific concern was whether the concomitant blockade of LTB_4 as well as cysteinyl leukotriene synthesis that results as a consequence of the blockade of 5-lipoxygenase might also prevent neutrophil chemotaxis. The system has proved to be more robust than feared, and there is no increase in significant respiratory infection when a 5-lipoxygenase agent is used compared with a leukotriene receptor antagonist that has no effect on LTB_4. Unfortunately, there is also no evidence that eosinophil chemotaxis is selectively diminished by LTB_4 inhibition resulting from synthesis inhibitors. Hence, there is no obvious therapeutic benefit to drugs that block the entire leukotriene synthetic pathway over receptor antagonists that specifically block the cysteinyl leukotriene receptor. A recently described phenomenon is the ability of the anti-leukotriene agents to block eosinophil migration, at least partially, even with short term use. In guinea pig tracheal explants, the chemotactic agent fMLP causes substantial and selective migration of eosinophils that reside naturally (and quiescently) in the lamina propria. Administration of zileuton in concentrations of $> 10^{-8}$ M caused substantial inhibition of eosinophil migration, and full blockade was achieved with higher concentrations[104]. Because LTB_4 receptors are significantly involved in the chemotactic process, it was postulated that the action of zileuton resulted from the unique ability of this anti-leukotriene to block the synthesis of LTB_4 as well as the SP1 analogues. The selective LTB_4 antagonist LTB_4 dimethylamide caused even more potent blockade of eosinophil migration in this model. However, administration of the highly selective LTD_4 receptor antagonist zafirlukast caused equipotent blockade. Significant inhibition of eosinophil chemotaxis was observed at 10^{-12} M zafirlukast. Comparable trials have been conducted in humans. Calhoun et al[105] have reported in a preliminary study that large doses of zafirlukast given over 24 hours caused an approximate 50% decrease in the migration of eosinophils into the airways of challenged asthmatic subjects, and Diamant et al[106] have shown independently comparable findings. The mechanism by which LTD_4 receptor blockade inhibits eosinophil migration remains elusive. It also remains to be determined whether the magnitude of inhibition of eosinophil migration in humans is clinically and pathophysiologically significant.

As asthma is viewed as an inflammatory process mediated at least in some part by leukotrienes, eosinophils are the major transport systems for these compounds to the airway smooth muscle where they cause contraction, and to the airway vasculature where they cause oedema. Leukotrienes are synthesised de novo in eosinophils directly from membrane phospholipids after activation by phospholipase A_2 (PLA_2). The process of selective chemoattraction is a fascinating one, as eosinophils are but a minor component of the circulating granulocytes. Even though eosinophils share common surface ligands with neutrophils, they are capable of selective migration into the airway wall. It is likely that cytokine specific processes regulate this selective migration for example, IL-5. It is also of considerable interest that the process of molecular adhesion and transmigration is intimately linked to the priming of eosinophil secretion of leukotrienes. The mechanism by which this occurs is unclear, but appears from some very preliminary studies to be related to the direct phosphorylation of PLA_2-IV, which may occur as a consequence of adhesion. Another property of leukotrienes that remains unexplained is the apparent ability of these compounds to cause, by a mechanism yet to be defined, substantial chemotaxis of eosinophils in both animal models and in humans. While eosinophils are the unique inflammatory cells of asthmatic inflammation, it is still unclear if they are essential for all manifestations of the asthma syndrome. It is further unclear whether leukotriene synthesis alone accounts for the bioactivity of these cells in causing airway narrowing in asthma. Blockade of leukotriene activity in human asthma does not cause improvement in airflow obstruction in a manner comparable to that obtained with corticosteroids or high efficacy b_2-adrenoceptor drugs. The invariable presence of eosinophils in human asthma does not itself imply a role for these cells in the pathogenesis of the disease. However, the demonstration that adhesion primed eosinophils are capable of causing massive augmentation of leukotriene secretion and that this secretion is of a magnitude sufficient

to cause contraction of human airway explants suggests that eosinophils are the source of leukotrienes in human asthma. Nonetheless, the roles of eosinophils and of leukotrienes in human asthma may vary among the different asthma phenotypes that are only now being defined.

Airway obstruction and airway hyperresponsiveness are important features of asthma and chronic obstructive pulmonary disease (COPD). Both diseases are characterized by airway wall and lung tissue inflammation, and in asthma there exists a relationship between the inflammatory state of the airways and the severity of hyperresponsiveness. However, the type and cause of this inflammation, as well as the extent and consequences of the inflammatory process, are different in asthma and COPD. Inflammatory processes affecting the airway wall both in peripheral and central areas of the lung appear to be important, the former one dominating in COPD and the latter in asthma. However, it is not clear which structural changes are open for therapy and which are not. Therefore, a better understanding of the consequence of inflammation for lung tissue and airway wall changes in asthma and COPD has to evolve before a full understanding of airway hyperresponsiveness will emanate. Airway hyperresponsiveness to nonspecific stimuli such as histamine, methacholine, and cold air is a hallmark of asthma, whereas many patients with COPD also have abnormal values in tests of hyperresponsiveness. Airway hyperresponsiveness is defined by an exaggerated response of the airways to nonspecific stimuli, which results in airway obstruction. It is yet unknown which factors within the airways of an individual are responsible for this exaggerated airway narrowing. Therefore, one could question why hyperresponsiveness is currently being assessed in research when this response is so nonspecific. Asthma and COPD differ with respect to maximal airway narrowing. There exists a limit to the response in COPD, i.e., a plateau in bronchoconstriction: no further narrowing occurs whatever the dose given. This plateau is lacking in more severe asthma. The most direct evidence of the relationship between excessive airway narrowing and airway inflammation comes from a study showing a correlation between the maximal response to methacholine and the eosinophil counts in bronchial biopsies in asthma. How far the above mechanisms contribute to this excessive narrowing has been studied to some extent but is not resolved as yet. Inflammation in central and peripheral airways can be assessed with sputum induction, bronchoalveolar lavage, airway wall biopsies, and transbronchial biopsies. They all reflect the inflammatory process, yet give different information. The changes that occur in the airways in asthma are caused by an influx of inflammatory cells with release of their mediators. This episodic and ongoing allergic inflammation may cause structural changes that affect the severity of airway obstruction after inhaling a bronchoconstrictor stimulus. Results of the inflammatory process in asthma are airway wall edema, deposition and remodeling of connective tissue components, hypertrophy and hyperplasia of tissue cells (for example, smooth muscles), and new vessel formation in the bronchial vasculature. These changes result in airway wall thickening, which may have a profound effect on airway function. This includes changes in thickness of airway wall areas, but also in stiffness of these areas due to biochemical changes of tissue. Structural changes in the airway wall, including extracellular airway remodeling, are prominent features of asthma. For instance, collagen deposition in the subepithelial matrix and hyaluronan and versican deposition around and internal to the smooth muscle can be present in asthma. These depositions can be expected to oppose the effect of smooth muscle contraction.

In COPD, the expiratory flow is influenced by the elastic properties of lung tissue, since elastolytic destruction of lung parenchyma decreases the elastic lung recoil and therefore the driving pressure for flow. Moreover, remodeling of airway wall tissue and airway smooth muscle contributes to the mechanical properties of the airway wall. Luminal secretions may increase resistance in the airways together with increased mucus gland size and goblet cell number and, finally, airway wall inflammation may change airway collapsibility as well. The increased airway collapsibility and loss of lung elasticity are irreversible, whereas the acute inflammatory changes may be open for therapeutic intervention. Acute deposition of proteoglycans and edema formation in submucosal and adventitial tissue may occur in COPD. This is potentially reversible. Chronic inflamma-

tion in COPD includes progressive collagen deposition and possibly changes in the balance of different proteoglycans and fibrosis of the airway wall. With the increase of airway wall thickness, mechanical properties change. Hyperresponsiveness can be assessed with a stimulus directly acting on the smooth muscle and a stimulus that induces smooth muscle contraction in an indirect way. The latter stimulus may activate either inflammatory cells or neural pathways, thereby enhancing airway obstruction. Methacholine and probably histamine are stimuli that directly act on the smooth muscle; cold air and adenosine-5-monophosphate (AMP) are indirectly acting stimuli. Methacholine challenge testing is one method of assessing airway responsiveness. Airway hyperresponsiveness is one of the features that may contribute to a diagnosis of asthma. It may vary over time, often increasing during exacerbations and decreasing during treatment with antiinflammatory medications. Methacholine challenge testing (MCT) is most often considered when asthma is a serious possibility and traditional methods, most notably spirometry performed before and after administration of a bronchodilator, have not established or eliminated the diagnosis. Symptoms that suggest asthma include wheezing, dyspnea, chest tightness, or cough in the following circumstances: (1) with exposure to cold air, (2) after exercise, (3) during respiratory infections, (4) following inhalant exposures in the workplace, and (5) after exposure to allergens and other asthma triggers. A history of such symptoms increases the pretest probability of asthma. The optimal diagnostic value of MCT (the highest combination of positive and negative predictive power) occurs when the pretest probability of asthma is 30-70%. Methacholine challenge testing is more useful in excluding a diagnosis of asthma than in establishing one because its negative predictive power is greater than its positive predictive power. Methacholine challenge testing is also a valuable tool in the evaluation of occupational asthma. Methacholine challenge testing is sometimes used to determine the relative risk of developing asthma, assess the severity of asthma, and assess response to asthma therapy although its clinical use in these areas has not been well established.

Viral Respiratory Infections and Airway Hyperresponsiveness

Viral respiratory infections induce airway hyperresponsiveness in asthmatic patients, in healthy persons, and in a number of animal species. In asthmatics the degree of airway hyperresponsiveness is associated with the severity of exacerbations. The respiratory tract of an asthmatic is inflamed, and these inflammatory cells might be involved in modulating airway responsiveness. In contrast, no data are available on the role of bronchoalveolar cells in the airways of “healthy” persons or asthmatic patients suffering a respiratory tract infection. Because of the lack of information on this issue, the present review has been written. A number of animal studies have now been performed suggesting the involvement of inflammatory cells during a viral respiratory infection. The changes in number and activity of bronchoalveolar cells after a viral infection have been compared with changes in airway morphology and the development of airway hyperresponsiveness. Based on these data we suggested, the following hypothesis: Viruses damage the epithelial layer of the respiratory tract and activate bronchoalveolar cells. Subsequently, a number of mediators are released that can stimulate metachromatic cells, which in turn release products that increase vascular permeability and attract inflammatory cells that might cause additional epithelial damage. Finally, the released mediators and the morphologic changes together results in airway obstruction and the development of hyperresponsiveness**.** A fuller understanding of the mechanisms of virus induced asthma exacerbations will hopefully lead to the identification of new potential targets for the development of novel therapeutic interventions. Rhinoviruses are not able to infect the lower airway, though there have been few data available to argue either way. In a recent study well designed to control for upper airway contamination, Gern et al.[107] used PCR to assess lower airway rhinovirus load during experimental infections. Bronchoalveolar lavage (BAL) cells were positive during the infection in over 80% of their samples, while only 37% of BAL fluid specimens were positive, suggesting that rhinoviruses are indeed able to infect the lower airway and that rhinovirus RNA was largely cell associated. A

further subject attracting considerable recent attention is the interaction between virus infections and allergy. It is known that simultaneous virus infection and positive specific IgE for inhalant allergens have a much higher odds ratio for the development of wheezing than any of the factors alone[108]. Schwarze et al used a murine model to show that respiratory syncytial virus infection not only produces airway hyperresponsiveness in the acute phase, but also subsequently enhances allergen sensitisation notably, pulmonary eosinophilic infiltration was observed in both situations[109]. An enhanced reaction to allergen inhalation in allergic patients experimentally infected with rhinovirus has also been demonstrated[110]. These data suggest that concurrent virus infection can increase the airway response to allergen (perhaps by increasing penetration of allergen through the damaged epithelium), but little is known about the reverse possible interaction. Effective virus clearance requires effective Th1 type responses. Asthma is clearly associated with Th2 type responses and might therefore be expected to be associated with less efficient virus clearance or more severe infections. Several studies have confirmed that asthmatic subjects have more severe symptoms during virus infections[111], but it is not known if the virus load or the residence time is greater. Induction of non-specific bronchial hyperresponsiveness is a feature of asthma and a well documented result of viral infection in allergic subjects[112,113], though this induction has not been observed in all studies[114]. This discrepancy in observations is probably related to differences in virus dose and/or in the inoculation method used[115]. Inflammatory cell infiltration is another important feature of asthma which has been observed with human experimental rhinovirus infection studies examining induced sputum (increased eosinophil products) or bronchial biopsy specimens (lymphocyte and eosinophil infiltrate) of asthmatic patients[116,117]. Inflammatory cells such as eosinophils, neutrophils, and lymphocytes, as well as the expression of intercellular adhesion molecule 1 (ICAM-1), were also found to be significantly increased in atopic subjects compared with non-atopic subjects experiencing natural colds[118]. Neutrophils are also implicated in the response to virus infection; their products have been observed in nasal secretions during wild type colds[119] and increased staining for IL-8 was observed in neutrophils in sputum during experimental rhinovirus infections. The cytokines regulating the inflammatory cell infiltrate in virus induced asthma and the mechanisms controlling their induction or release are clearly of great current interest. Interleukin 8 (IL-8) has been implicated in the pathogenesis of respiratory virus infection and asthma. Rhinoviruses were found to induce IL-8 release from monocytes for up to a week after inoculation onto peripheral blood mononuclear cells[120]. While some respiratory viruses are able to replicate inside monocytes and macrophages, this does not seem to be the case with rhinoviruses[121], even though low grade replication was shown in a human monocyte cell line. The function of IL-8 as a chemotactic factor for neutrophils and primed eosinophils indicates that it may play an important part in triggering the inflammation that leads to exacerbations of asthma. Indeed, IL-8 has been detected in increased amounts in both nasal and bronchial samples taken during virus infections, and levels correlated with induction of bronchial hyperreactivity. Likewise, the proinflammatory cytokine IL-6 was found to be induced by rhinoviruses[122], and IL-6 was also increased in the sputum of rhinovirus infected asthmatic subjects[116]. Another recent candidate which may be important in the pathogenesis of virus induced asthma is IL-11. Several viruses including respiratory syncytial and parainfluenza viruses and rhinovirus strongly stimulated its production by stromal cells. Furthermore, it was detected in nasal aspirates from children with upper respiratory infection and its levels were correlated with clinically detectable wheezing[123]. With regard to the intracellular mechanisms of cytokine induction, factors binding to the NFB transcription factor binding site on the IL-6 promoter were shown to be involved[122], but it is not yet known whether blocking the activity of this transcription factor is sufficient to inhibit virus induction of proinflammatory cytokines. Such interesting observations on the epidemiology, diagnosis, and pathophysiology of virus induced asthma are beginning to suggest new candidate molecules that might represent targets for novel therapeutic interventions. It is hoped that further advances in our under-

standing of the cellular and molecular mechanisms involved will lead to more clear identification of such targets, and the development of blocking strategies suitable for testing in the clinic. In the meantime, we will have to continue to use increased doses of inhaled or oral steroids until more effective antiviral therapies are available[124].

Hyperresponsiveness and Genetic Control

Hopp et al reported that hyperresponsiveness to methacholine is under genetic control with a heritability of 66%[125]. The clinical characterization of asthma is difficult, and this complicates the mapping of genes for the disorder. However, critical components of asthma, such as bronchial-hyperresponsiveness and allergic status, can be defined more objectively. Linkage analysis was used to identify a genetic location for bronchial hyperresponsiveness. Candidate regions for atopy have been described through linkage analyses. Previous studies have suggested that atopy and bronchial hyperresponsiveness in the current study population are not linked to a genetic locus on chromosome 11q. However, there is evidence of a major gene for atopy on chromosome 5q31-q33. Therefore, to determine the chromosomal location of a gene or genes governing susceptibility to bronchial hyperresponsiveness, which would be coinherited with a major gene for atopy, linkage analyses between bronchial hyperresponsiveness and genetic markers on chromosome 5q were performed. Analyses of affected pairs of siblings demonstrated statistically significant evidence of linkage between bronchial hyperresponsiveness and D5S436, D5S658, and several other markers located nearby on chromosome 5q31-q33. These data strongly support the hypothesis that one or more closely spaced genes on chromosome 5q31-q33 determine susceptibility to bronchial hyperresponsiveness and atopy[126].

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