REVIEW

The distal airways: are they important in asthma?

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The distal airways: are they important in asthma? M. Kraft. © ERS Journals Ltd 1999.
ABSTRACT: Although the airways of <2 mm in diameter have been dubbed the "quiet zone", they do not appear to be so in asthma. Physiological and pathological evidence suggests that the small airways and lung parenchyma participate in asthma pathogenesis, and may explain many of the clinical observations noted.

This review presents this evidence, beginning with physiological evidence, followed by pathology and last by imaging studies that evaluate the distal lung. Seminal physiological studies date back to the 1960s, with significant progress in the area of airway smooth muscle and its contribution to airways responsiveness noted over the last several years.

The use of bronchoscopy in clinical studies has complemented the autopsy studies in advancing knowledge about airway structural changes appreciated in asthma in the small airways and lung parenchyma. These pathological studies have allowed validation of the physiological, and more recently the imaging studies performed to evaluate this compartment of the lung in asthma.

Thus, the evidence suggests that the small airways and parenchyma contribute significantly to asthma pathogenesis. The challenge now lies in evaluating this compartment in the context of its value as a therapeutic target in asthma.
Eur Respir J 1999; 14: 1403–1417.

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Data from the last three decades have suggested that the distal lung, which includes the airways of <2 mm and the lung parenchyma, contributes to asthma pathogenesis. Due to the challenges raised in evaluating this part of the lung, this region has not been studied in the same level of detail as have the larger airways. However, a significant amount of pathological data from autopsy specimens and recently from chronic, stable asthmatics, evaluating the small airways and lung parenchyma, are available. These data, combined with physiological data, support a significant role for the distal lung as a contributor to airway inflammation and hyperresponsiveness. The following review will present this evidence and explore the clinically important issue of the distal lung as a therapeutic target in asthma.

Physiology of the distal airways

Animal and *in vitro studies*

The small airways of <2 mm in diameter are pathways of low resistance, and normally contribute ~10% of the total resistance to flow. These observations were made in, seminal contributions by MACKLEM and MEAD [1, 2] using a retrograde catheter technique. Using excised dog lobes, they placed a catheter containing bell shaped polyethylene tubing at one end. The bell was wedged into a bronchus with the catheter extending peripherally through the parenchyma and pleura. They showed that the peripheral resistance (R_p) was too small to detect at >80% of vital capacity (VC), but increased at lower volumes to 15% of the total lung resistance at 1% of VC. These data are in keeping with WEIBEL [3], who demonstrated that the cross-sectional area of the small airways was significantly larger than that of the central airways.

BROWN *et al.* [4] further evaluated the effects of small airways occlusion on total lung resistance by placing small beads into the peripheral airways of both dogs and pigs to block the small airways, and occluded the larger airways with larger beads. These animal models were chosen as dogs have collateral channels, or communications between alveoli and bronchioles, whereas pigs do not. In the dog model, gas was still able to reach the alveoli, despite occlusion of the airways, by diffusion through collateral channels. This occurred without a change in VC. In the pig model, without collateral channels, occluding the small airways produced significant reductions in VC and ventilation. The VC was reduced by 50% as 50% of the alveoli did not ventilate. This experiment demonstrated that if collateral ventilation is present, obstruction of the small airways has very little effect on mechanical properties of the lung. However, it does affect distribution of inspired gas.

HOGG *et al.* [5] confirmed this concept using a single breath nitrogen washout curve following an inflation with 100% oxygen. Before the beads were placed in the airways of dogs as described above, the nitrogen concentration on the alveolar plateau was flat; that is, the alveolar gas distribution was uniform and ventilation was evenly distributed. After the beads produced small airways obstruction, alveolar gas composition became nonuniform, with rising nitrogen concentrations on the alveolar plateau. The early expired gas had a low nitrogen concentration suggesting that it was from well ventilated alveoli. The later gas, with higher nitrogen and lower oxygen concentrations, came from collaterally ventilated spaces. These experiments helped to confirm that in species with collateral ventilation such as man, small airway obstruction

may have little effect on typical lung mechanics, but does effect ventilation distribution.

Although asthma was not specifically addressed in their study, Hogg et al. [7] used the retrograde catheter technique in *post mortem* specimens from patients with emphysema and showed that the R_P was increased 4–40 times compared to normal lungs. They also measured central airways resistance, and found that it was also increased, but variably in those patients with emphysema. Of interest, peripheral resistance was increased in some lungs without a concomitant increase in total lung resistance. The authors speculated that an individual who experiences chronic cough and sputum production may have considerable small airway obstruction and an increase in R_P with near normal total airway resistance. Thus, the airways were dubbed the "quiet zone" of the lungs by J. Mead in 1970 [8, 9].

These results were challenged by VAN BRABANDT et al. [10]. They repeated the experiments of Hogg et al. [7] described above, in normal lungs and compared the results to those in lungs with chronic obstructive disease. They used the retrograde catheter technique, but then oscillated the lung with a complex signal frequency as used by Hogg et al. [7]. Via a Fourier transformation, they were able to determine the resistance at 16 frequencies (2–32 Hz). They found that in normal lungs as well as those with chronic obstructive disease, the peripheral resistance contributes 50–90% of the total resistance between the main bronchus and pleura. The central resistance, as measured between the main bronchus and airways 2–3 mm in size, is modified only slightly in the presence of obstructive lung disease. They concluded that the peripheral resistance is underestimated and that in fact the small airways are anything but quiet. Of importance is that the resistance measured is dependent on the frequencies used to measure the resistance. Lower frequencies, which were used by VAN BRABANDT et al. [10] reach the small airways more effectively than the frequency used by Hogg et al. [7] (15 Hz), and thus revealed an increased contribution of the peripheral airways and tissue to total airways resistance. Therefore, these studies cannot be directly compared.

The studies described above illustrate that factors leading to small airway disease in chronic airflow limitation are due to unique anatomical and physiological differences between the large and small airways, as well as the influence of the lung parenchyma. Firstly, the small airways are less able to clear secretions due to a lack of cilia and a lack of high gas velocity generated during cough. Normally, this is not a significant issue, but in asthma and other obstructive lung diseases where small airway inflammation and goblet cell metaplasia occurs [11, 12], these factors become magnified. In addition, MICHEL et al. [13] have shown that pollen particles reach the small airways and alveoli, which may further increase the inflammatory response in the atopic asthmatic patient if clearance mechanisms in this part of the lung are not adequate. Given the small radius of curvature and high compliance of the small airways as demonstrated by MARTIN and PROCTOR [14] these factors can lead to instability and airway closure at low lung volumes. In this setting, surfactant may confer stability by protecting against excessive change in radii with changes in volume. This possibility was suggested by MACKLEM and MEAD [1] and demonstrated in a subsequent study in cats [12]. In asthma, surfactant may be replaced by inflammatory exudate or mucus, thus rendering the small airways prone to obstruction and closure. In fact, surfactant dysfunction has been described in asthma [15, 16]. If the stability of the peripheral airways were lost due to increased secretions and/or surfactant dysfunction, these events could lead to narrowing of the peripheral airways, airway closure, higher lung volumes and gas trapping.

In addition to the factors discussed above, the effectiveness of cough and secretion clearance also depends on the equal pressure point (EPP). The EPP is the point or points along the airway where at maximum expiratory flow, the pressure at the inner wall of the airways is equal to the pleural pressure. The EPP divides the airway into upstream segments between the alveoli and the EPP, and downstream segments between the EPP and airway opening (mouth) [2]. By examining expiratory flow after subjects breathed a helium-oxygen mixture, DESPAS et al. [17] showed that in subjects with asthma with small airways obstruction, the EPP was most likely situated where the total cross sectional area is large and the flow upstream is laminar and fully developed. The rationale for this approach is that fully developed laminar flow is independent of gas density. If expiratory flow is increased with a gas mixture such as helium where the density is decreased compared to oxygen, this suggests that the flow is not laminar. As the small airways are the only site where fully developed laminar flow exists [17], an increase in expiratory flow due to helium suggests large airway obstruction. However, an increase in expiratory flow does not exclude small airways obstruction, but indicates that the large airways with higher Reynold's numbers are involved to a greater extent in bronchoconstriction than when the helium response is not present. In normal subjects, the cross sectional area at equal pressure points approximates to the trachea. Therefore, maximum expiratory flow is density dependent, *i.e.* increases after inhalation of helium, as laminar flow is not fully developed in the large airways.

Further evaluation of the distal lung was examined in a canine model by LUDWIG and coworkers [18–23]. They evaluated the dose response behaviour of canine airways and parenchyma employing histamine [18]. Airway resistance, (R_{aw}), was measured by determining the pressure at the end of a catheter wedged into a subsegmental bronchus that delivered air and histamine at specific flow rates. Resistance of the parenchyma, termed tissue viscance, was measured using a device called an alveolar capsule [24]. The alveolar capsule, containing a pressure transducer, was placed on the outer pleural surface, puncturing the pleura. This allowed the pressure that developed in the parenchyma, generated by the flow of gas through the tracheal catheter, to be measured. The measurement of tissue viscance requires a measure of alveolar relative to pleural pressure in phase with flow. As these studies are read, it is important to note that this measurement is dependent upon the tidal volume and cycling frequency [1, 25–28]. As discussed above, the magnitudes of airway resistance and tissue viscance are extremely sensitive to how the measurements are made. High frequency, low amplitude oscillations at low lung volumes will minimize the contribution of the tissues to total resistance; low frequency, high amplitude oscillations at

higher mean lung volumes will maximize the contribution of peripheral airways resistance, which includes tissue viscance, to the total lung resistance. The studies described employing the alveolar capsule technique used tidal volumes in the physiological range and low frequency oscillations to mimic tidal breathing. The investigators used low frequency (0.3 Hz) and tidal volumes of ~300 mL to minimize nonhomogenous distribution of airflow [29, 30]. These issues are important as measurements of tissue viscance are made in one area of the lung with results extrapolated to the whole lung.

Using this technique, LUDWIG et al. [18] found that tissue viscance, a function of air flow through the catheter, pressure in the alveolar space and lung elastance, accounted for 78±8% (mean±SD) of total resistance under baseline conditions. Although the proportion remained constant during the histamine dose response curve, the absolute total lung resistance, tissue viscance and airways resistance increased with increasing doses of histamine (fig. 1). The percentage change in tissue viscance correlated significantly with the percentage change in airway resis-tance (r=0.77, p<0.001).

A similar finding was demonstrated in rabbits, where tissue viscance contributed to 64.5±15.9% (mean±SD) of total lung resistance at baseline and 83.6±11.8% after methacholine induced bronchoconstriction [20]. The changes in tissue viscance may reflect some modification in the intrinsic properties of the lung tissues [18, 28] and suggest that the parenchyma may exhibit contractile properties [31]. The quick resolution with atropine and the lack of histological evidence for interstitial or alveolar oedema make the presence of extravascular lung water a less likely explanation. Therefore, contractile responses may be occurring in both the airways and the parenchyma.

The question of whether the parenchyma exhibits true contractile responses or whether they are just a consequence of airway closure and/or extravascular lung water has been explored by FREDBERG et al. [32]. Using isolated parenchymal strips from male guinea pigs, FREDBERG et al. [32] demonstrated that the parenchyma can express diverse responses to several agonists such as histamine, prostaglandin D_2, leukotriene C_4, methacholine, and sub-stance P. The responses to these agonists varied in intensity but were present and significant. These responses suggest that the parenchyma exhibits contractile responses probably due to the presence of smooth muscle, which may further contribute to the physiological responses of the distal airways.

Whether the site of the contractile response is at the alveolar level is controversial. Potential mechanisms include contraction of myofibroblasts, contractile interstitial cells or constriction of the alveolar duct and/or small airway smooth muscle [33–35]. TEPPER and coworkers [36, 37] have shown that oscillation itself can affect contractile responses in a smooth muscle preparation. DOLHNIK-OFF et al. [38] evaluated subpleural parenchymal strips from humans to determine dynamic measures of resistance, elastance and hysteresivity. Hysteresivity, defined by FREDBERG and STAMENOVIC [39], is a parameter used in the assessment of lung tissue behaviour, one in which energy dissipation and storage are linked. Hysteresevity is a function of tissue viscance and lung elastance and is modulated by the contractile state of contractile cells distributed throughout parenchymal tissue. Hysteresevity has been shown to increase with the administration of methacholine in a rabbit model described above by ROMERO et al. [21]. DOLHNIKOFF et al. [38] revealed that stimulation of human lung parenchymal strips with acetylcholine resulted in an increase in tension, resistance, elastance and hysteresivity. The response between strips was similar, and did not depend on the number of small airways present within the strips. They also evaluated the presence of smooth muscle actin by immunohistochemistry and found actin positivity in the small airways, alveolar ducts and alveolar walls. Thus, these data are consistent with other studies [18, 32] but these investigators took the additional step of suggesting that the contractile elements are at the level of the alveolar wall.

Human studies

Studies evaluating the function of the distal airways in humans range from the noninvasive evaluation of dynamic compliance and airway hysteresis to direct measurement of peripheral airways resistance *via* bronchoscopy [40–49]. WOOLCOCK et al. [40] determined the dynamic compliance in four subjects with asthma, five subjects with chronic bronchitis and eight normal controls. This was performed by first measuring static compliance *via* an oesophageal balloon, then asking subjects to breathe at increasing frequencies up to 120 breaths·min^{-1}. Dynamic compliance was calculated by dividing the volume change by the pressure change between points of zero flow on the tracing. In subjects with asthma and chronic bronchitis, there was a significant frequency dependence of dynamic compliance, particularly in the asthmatics, where dynamic compliance fell to ≤25% of static compliance at the highest frequency. After isoproterenol, less frequency dependence was appreciated particularly in the asthmatics, but in no case did compliance become independent of frequency. Thus, the frequency dependence suggests that some regions of the lung are filling and emptying at different rates than other regions, which can produce ventilation-perfusion mismatching. These data support conclusions by MACKLEM and MEAD [1], who showed that the time constants of the lung units distal to the
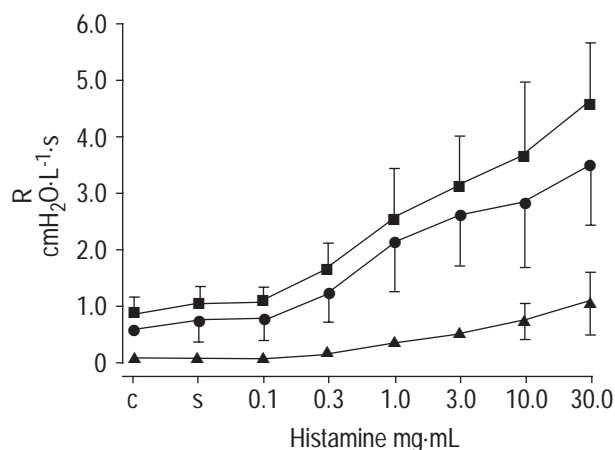

Fig. 1. – Resistance (R) values for ■: total lung resistance; ▲: airways resistance; ●: tissue viscance under control conditions and after each concentration of histamine. Values are mean±SEM (n=6). c: control; s: saline. (Adapted from [18]).

airways <2 mm were in the order of 0.01 s. They determined that a four-fold difference in these time constants between lung units, which can occur secondary to inflammation, goblet cell metaplasia and/or narrowing due to collagen deposition would be sufficient to cause dynamic compliance to fall with increasing respiratory frequency [1]. Of importance is the fact that these changes occurred in asthmatics whose spirometry was within the normal range.

A method to measure intrabronchial pressure directly in humans to determine central and peripheral airways resistance was performed by YANAI et al. [50]. This method is comparable to the retrograde catheter technique discussed above, in that the catheter tip was wedged into a 3 mm bronchus and the airway lateral pressure (central resistance (Rc)) just proximal to the wedged bronchus was measured. They determined Rp as the difference between total lung resistance and the central resistance. However, these studies were performed in humans with asthma, and compared to the peripheral resistance measured in excised lung from five dogs similar to the study by HOGG et al. [7] with a correlation of 0.99. They showed that the peripheral resistance was increased in asthma as compared to control subjects, whereas Rc showed a tendency to increase in asthma, but not significantly so. The increase in Rp was more dramatic, and was also increased as compared to central resistance in those subjects with chronic bron-

chitis and emphysema. Overall, the contribution of small airways and tissue to the total lung resistance was ~15%. It could be argued that measurement of resistance in a single lobe of the lung is not indicative of total lung resistance. However, these values were similar to those described by HOGG et al. [7], who measured resistance throughout post mortem human lung specimens.

The use of volume history response in humans can also shed light on parenchymal hysteresis, a reflection of tissue viscance. Hysteresis refers to the fact that more transmural pressure is required to keep the airway at a certain dimension (and volume) on the inflation limb as compared to the deflation limb (fig. 2). The lung parenchyma and the large airways are highly interdependent mechanically, such that changes in the size of each occur simultaneously [51] and each also has hysteresis. Most of the pressure volume hysteresis is due to the parenchyma, which in this setting also includes the terminal airways and is attributable to the surfactant acting at the gas-liquid interface [42]. In addition, there is an effect on hysteresis produced by opening and closing of the extremely peripheral airways which is related to intrinsic tone of small airway smooth muscle and contractile elements of alveolar ducts [42]. Thus, parenchymal hysteresis (which includes the terminal airways), is the tissue viscance described above by LUDWIG et al. [18].
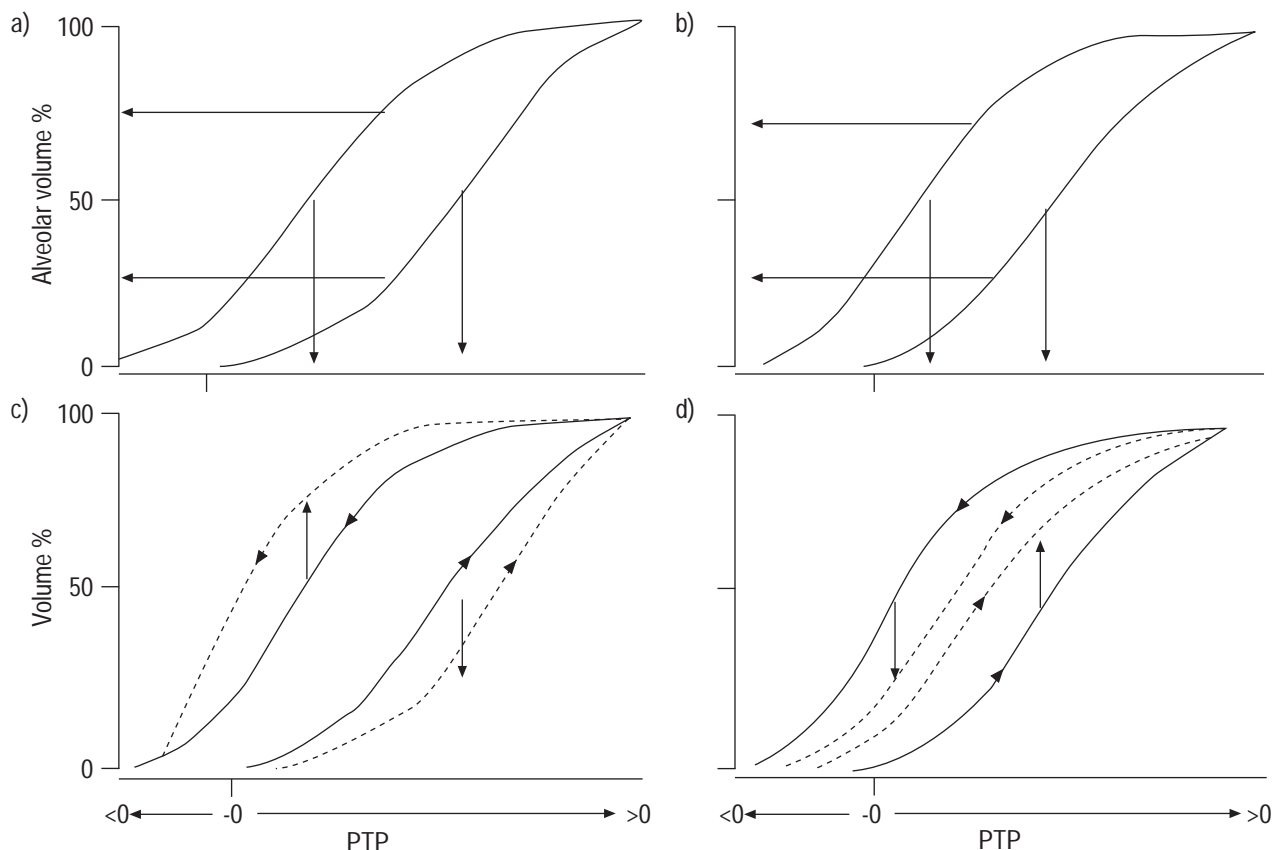

Fig. 2. – All four panels plot normalized volume (a) of alveoli alone; b) of airways alone; c) and d) alveoli as solid lines and airways as dashed lines) versus transpulmonary pressure (PTP). Arrows on lines show direction of volume change. It is assumed that PTP equals transmural pressure of airways as well as alveoli (parenchyma). Horizontal lines in a) and b) indicate the volume difference at the same PTP showing a higher volume during deflation. Vertical lines indicate pressure difference at a given volume, showing a lower PTP at the same volume during deflation. c) shows airway (dashed line) hysteresis relatively greater than parenchymal (solid line) hysteresis. With airway size as the dependent variable, c) shows airway hysteresis greater than that of the parenchyma and indicates that airways are larger at a given lung volume after a deep inflation. d) shows airway hysteresis less than that of the parenchyma so that following a deep inhalation, airways are smaller at a given lung volume. (Adapted from [42].)

Hysteresis of the airways at all levels throughout the tracheobronchial tree is directly proportional to airway smooth muscle tone, with increased tone associated with increased hysteresis, and decreased tone associated with decreased hysteresis [52]. FROEB and MEAD [51] have provided a method that illustrates how relative differences in hysteresis of the airway, which refers to the large airways and parenchyma, which refers to alveolar tissue and the terminal airways, could account for this variability in volume-history response. Three patterns exist when airway size, or volume, is plotted against lung volume. When airway hysteresis exceeds parenchymal hysteresis, airway size is greater on the deflation limb than it is on any given volume of the inflation limb. When airway hysteresis is less than parenchymal hysteresis, there is smaller airway size during deflation than during inflation. If airway and parenchymal hysteresis are the same, volume history has no effect on airway size.

The use of deep inhalation and the ratio of maximal to partial flow rates at the same volume illustrates the concept of airways and parenchymal hysteresis [42, 43] (fig. 3). A maximum to partial (M/P) flow ratio >1 means that flow increased after a deep inhalation, suggesting that airway size is greater after a deep inhalation and airway hysteresis exceeds that of the parenchyma. Therefore, a deep inhalation produced bronchodilation. A low M/P ratio indicates that parenchymal hysteresis exceeds airway hysteresis, thus large airway size will decrease after a deep inhalation. In this case, a deep inhalation produced bronchoconstriction.

An observation that may shed light upon these disparate responses to deep inhalation has been shown in patients with mild asthma. When airway obstruction was induced by hyperventilation with cold air [53], methacholine [41] or histamine [54], a deep inhalation most often resulted in lessening of obstruction that is transient (bronchodilation). The degree of improvement was proportional to the degree of induced obstruction. This observation is compatible with an increase in airway hysteresis and is possibly due to constriction of large airway smooth muscle. In contrast, when the airway obstruction is spontaneous, a deep inhalation results in bronchoconstriction, opposite to the effect seen in induced obstruction. The hypothesis

suggested by Ingram is that the response to a deep inhalation might be indicative of the degree of peripheral inflammation. PLISS et al. [55] assessed the volume history response and bronchoalveolar lavage (BAL) eosinophils, histamine and protein. As the M/P ratio decreased, indicative of increased parenchymal hysteresis, the BAL eosinophils, protein and histamine increased. Other explanations for this observation may be stimulation of contractile elements in the arenchyma as discussed above by DOLHNIKOFF et al. [38] and FREDBERG et al. [32] and reduction of the forces of interdependence between airway smooth muscle and lung parenchyma by transudation of oedema or inflammatory exudate into the peribronchial space. Therefore, the response to a deep inhalation may be helpful in determining the balance of airway and parenchymal hysteresis, and may determine which process dominates. If a deep inhalation results in bronchodilation, then large airway hysteresis dominates. If it causes bronchoconstriction, parenchymal and small airway hysteresis dominates.

The mechanism driving the effect of deep inhalation in both asthmatic and nonasthmatic subjects has been explored by FREDBERG and coworkers [56–58] in several important studies. Long before the studies discussed above, FISH et al. [59] observed that airway obstruction in asthma behaves as it were caused by an instrinsic impairment of the bronchodilating properties of a deep inspiration. This observation is supported by the loss of the dose response plateau appreciated in asthma when smooth muscle agonists such as methacholine are administered [60]. Interestingly, this loss of bronchodilation with deep inspiration can be produced in normal, healthy subjects by asking them to voluntarily refrain from taking deep inspirations. Within 15 min, the airways become hyperresponsive to a degree that is indistinguishable from asthmatic subjects [61]. Several hypotheses have been suggested to explain these observations: there is greater smooth muscle mass in the asthmatic airways, such that a greater degree of narrowing results for a given degree of muscle stimulation [62, 63]; airway wall thickening caused by airway remodelling and/or cellular infiltration amplifies airway narrowing [64, 65], the asthmatic smooth muscle shortens excessively as a result of decreased airway elastance or
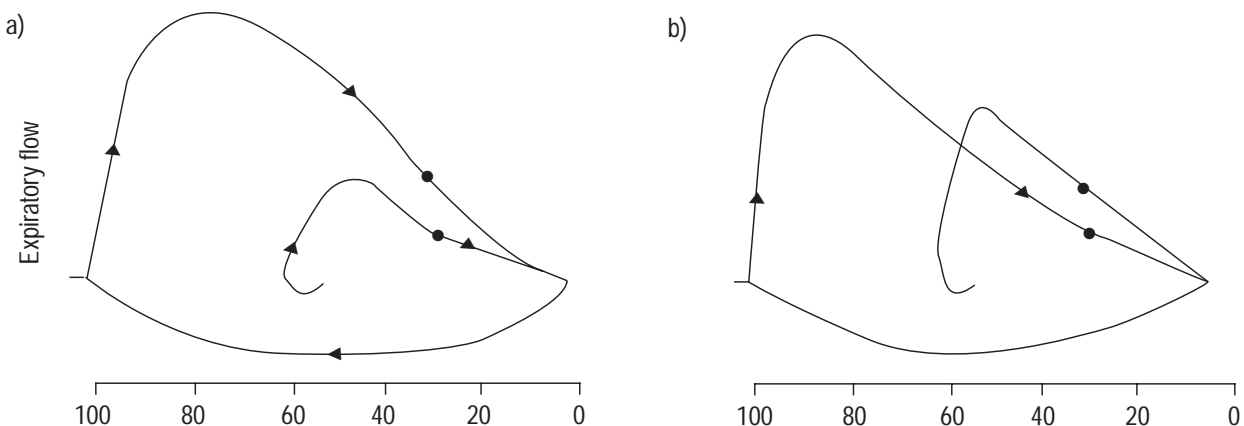

Fig. 3. – Representative forced exhalations, first begun from ~60% of vital capacity (VC) (partial manoeuvre), and quickly followed by inhalation of total lung capacity (plotted at 100% VC) before a second forced exhalation (maximal manoeuvre). At ~30% VC maximal (M) and partial (P) expiratory flows were compared to give M/P ratios. a) the M/P ratio is >1.0, indicating bronchodilation to a deep inhalation. The reverse is shown in b), where M/P<1.0. Arrowheads indicate the direction of airflow. Circles indicate the flow at 30% VC. (Adapted from [42].)

reduced mechanical interaction with the lung parenchyma [66] or that the smooth muscle in asthma is intrinsically different from nonasthmatics [67].

The latter hypothesis is particularly intriguing in that FAN *et al.* [68] have shown that tracheal muscle strips isolated from ovalbumin sensitized mice exhibit increased hyperresponsiveness and increased maximal shortening velocity without changes in isometric force generation. Allergen sensitization has been shown to increase the total activity and quantity of myosin light chain kinase, which is felt to increase the ability of actin and myosin to form crossbridges [69]. These observations are in accordance with the hypothesis brought forth by FREDBERG *et al.* [57], who have shown that tidal breathing, by virtue of muscle stretch, perturbs the binding process of myosin and actin, such that the bridges that they create detach much sooner than they otherwise would have and decrease the fraction of the time that the myosin is attached to the actin filament. Therefore, tidal breathing decreases the myosin duty cycle, which implies fewer myosin bridges, and decreased muscle stiffness. The more the muscle stretches, the more compliant it becomes, and therefore, the easier it is to stretch. These ideas comprise the perturbed equilibrium hypothesis [57].

This hypothesis certainly has implications in the asthmatic airway, in that the force fluctuations created by tidal breathing are subject to factors that lessen peribronchial distending stress, such as thickening of peribronchial adventitia, loss of elastic recoil, breathing at low lung volumes or failure to take deep breaths [70, 71]. If any or all of these factors are present, the muscle would stretch less with each breath, and become progressively stiffer such that physiological forces acting on the muscle would become insufficient to stretch the muscle appreciably. Bronchial hyperresponsiveness may be due to the failure of the underlying perturbed equilibrium to sustain itself.

Furthermore, the problem in asthma may not be that the muscle is too strong, but rather too fast. Muscle that cycles quickly, *i.e.* creates cross-bridges quickly, may be less likely to be perturbed by tidal breathing, as it can reattach and contribute once again to active force and stiffness. Combining all these factors, this hypothesis suggests that asthma is a process that results in inflammation driven remodelling of airway connective tissues and modification of the processes that regulate cross bridge cycling rates [68, 71, 72].

To further explore the airway-parenchymal interaction, an illustrative study was performed by KAMINSKY *et al.* [48]. They showed that hyperpnoea caused significant changes in the airway parenchymal interaction. They measured specific conductance, static compliance, lung volumes and M/P ratios before and after 5 min of hyperpnoea in seven asthmatics and six normal controls [48]. Hyperapnoea caused a fall in specific conductance and an increase in the M/P ratio thought to be due to large airway smooth muscle contraction, hyperpnoea in asthmatics also caused an increase in residual volume and pressure volume hysteresis, indicative of changes in parenchymal lung mechanics. The increase in residual volume may be secondary to airway closure, as demonstrated by GUNST *et al.* [73]. They showed that after maximal bronchoconstriction by methacholine in excised dog lobes, airway closure occurred at transpulmonary pressures of $7.5–10$ cmH$_2$O. The forces of parenchymal interdependence were not sufficient to prevent airway closure.

Furthermore, response to bronchodilators can also shed light on the contribution of airways and parenchymal hysteresis in asthma [42, 44]. WANG *et al.* [44] determined maximal and partial flow volume loops before and after progressive doses of albuterol ($180–5,040$ µg, cumulative dose). As an inhaled bronchodilator effects primarily the large conducting airways at conventional doses, M/P ratios initially fell as airway hysteresis decreased, and therefore parenchymal hysteresis dominated. However, in some subjects, the M/P ratio initially fell, suggesting a fall in airway hysteresis but then increased at the highest doses of albuterol administered. This response suggests a reduction in parenchymal hysteresis was thought to be secondary to systemic absorption of the bronchodilator. The authors concluded that hysteresis analysis can explain the effects of bronchodilator on large and small airway calibre.

The resistance of the peripheral airways, including the small airways and parenchyma, has been measured directly in humans using a variation of the retrograde catheter technique described by MACKLEM and MEAD [1]. In a study by WAGNER *et al.* [45], a bronchoscope was wedged into the right upper lobe of asthmatic and control subjects. A catheter administering warm, humidified air with 5% CO$_2$ at flow rates ranging from $100–500$ mL·min^{-1} was placed through the suction port. A pressure transducer at the end of the catheter measured pressures with each flow rate at functional residual capacity. The R_P was defined as the pressure divided by flow averaged over at least three flow rates. Using this technique, WAGNER *et al.* [45] showed that in asthmatics with normal spirometry, R_P was increased up to seven-fold as compared to control subjects (fig. 4). Furthermore, conductance (l/R_P) generally correlated with methacholine. The latter finding is particularly interesting, given the usual disparity between airways inflammation and bronchial hyperresponsiveness [74]. Potential mechanisms driving this relationship are inflammation, oedema and secretions which can produce a decrease in the baseline radius of the small airways. These changes may extend to the collateral communications and alter the forces of interdependence [8].

WAGNER *et al.* [46] extended these observations by assessing small airways hyperresponsiveness by directly administering histamine at 10, 50 and 100 mg·mL^{-1} and isoproterenol at 2 mg·mL^{-1} to the small airways using the wedged bronchoscope technique. Again, baseline R_P was greater in asthmatics with normal spirometry as compared to control subjects (p=0.019). More histamine was required in control subjects to cause a 100% increase in R_P (log provocative concentration causing 100% increase in R_P (PC$_{100}$)) than in asthmatics (p=0.0114). In asthmatics, the PC$_{100}$ correlated with whole lung responsiveness to histamine (r=0.847, p<0.05). Isoproterenol completely reversed the increase in R_P in control subjects, but not in the asthmatic subjects. This may be due to decreased drug delivery, decreased β$_2$-receptor number and/or decreased receptor binding affinity.

The mechanism by which histamine primarily increases peripheral airways resistance in asthmatics and to a small degree in control subjects merits discussion. Histamine has been shown to act directly on airway smooth muscle of the small airways in control subjects *in vitro* [75]. Histamine (H1) receptors have been shown on airway smooth muscle with the same distribution in asthmatics and nonasthmatics [76]. Although histamine can cause increased fluid

extravasation and oedema, this was not felt to be the mechanism of increased peripheral resistance based on evidence by BERMAN et al. [49]. Using a similar technique to that described above by WAGNER et al. [46], they instilled bradykinin in the small airways of asthmatics and control subjects. They showed a dose dependent increase in R_p in asthmatics, but not in control subjects. However, the amount of protein extravasation, thought to be a marker of airway oedema, was the same in both asthmatics and control subjects. The authors hypothesized that increased responsiveness of the small airways in the asthmatics was not due to bradykinin or histamine induced fluid extravasation, but possibly through a direct effect on airway smooth muscle or stimulation of sensory neural reflexes.

Peripheral airway dysfunction may also contribute to exercise induced bronchospasm. KAMINSKY et al. [47] measured R_p in asthmatics and control subjects before and after cool, dry air was delivered to the small airways for 5 min using the wedged bronchoscopic technique described above. Only the asthmatics exhibited an increase in R_p following the cool air challenge which inversely correlated with the change in forced expiratory volume in one second (FEV1) after exercise (r=-0.76, p=0.03), suggesting that both large and small airway changes occur with exercise.

The site of airway obstruction in asthma may have clinical implications. Building upon the work by DESPAS et al. [17], ANTIC and MACKLEM [77] assessed the effect of breathing a helium-oxygen mixture on the maximal expiratory flow volume curve. By evaluating expiratory flows before and after an inhaled β_2-agonist, they found that asthmatic subjects who smoked or experienced recurrent respiratory infections were more likely to exhibit significant obstruction of the small airways, as illustrated by lack of expiratory flow increase after helium. The asthmatic who did not smoke or experience recurrent infections was more likely to exhibit obstruction of the large airways. As discussed above, the improvement in expiratory flow seen after helium does not rule out small airway obstruction, it suggests that large airways obstruction is also present.

As another method to assess small airways involvement in asthma, SEKIZAWA et al. [78] assessed the methacholine dose response curves in control and asthmatic subjects. By simultaneous measurement of respiratory resistance and anatomic dead space through the use of oscillometry (5 Hz) and CO_2 as a test gas, respectively, the latter as described by LANGLEY et al. [79]. Methacholine was continuously inhaled in stepwise incremental concentrations during tidal breathing. A large airway responder was defined as those subjects who experienced a significant increase in anatomic dead space with methacholine, whereas a small airways responder was defined as no significant increase in anatomic dead space but significant increases in total resistance. The small airway responders had significantly greater reactivity than the large airway responders. The conclusion is that the site of airway response is one of the factors that determines the severity of bronchial hyperresponsiveness in asthma.

Pathology of the distal lung units

Inflammation in the distal lung units in asthma has been assessed primarily via autopsy studies and surgical speci-
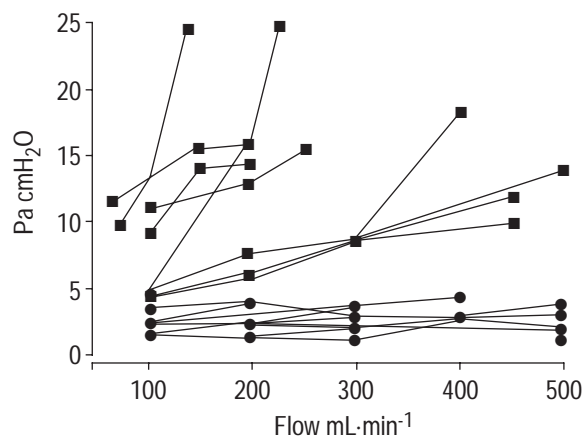

Fig. 4. – Pressure (Pa)-flow relationships of control subjects (●) and subjects with asymptomatic asthma (■). (Adapted from [45].)

mens. Recently, there have been three studies published where transbronchial biopsies have been performed in chronic, stable asthma [80–82]. Most of the studies from autopsy and surgical specimens focus upon the large and small airways, whereas the studies employing transbronchial biopsy describe lung parenchymal inflammation. To review these studies, this section has been divided into studies addressing airway structure and studies specifically addressing inflammatory cells.

Airway structure

The studies described below are shown in table 1. KUWANO et al. [83] compared small airway dimensions in fatal asthma, nonfatal asthma, chronic obstructive pulmonary disease (COPD) and control subjects. Fifteen subjects were in each group, and smoking status of the asthmatic group was not known. Several parameters were examined, including the adventitial perimeter, which was defined as the border between the peribronchial space and surrounding alveolar spaces, the smooth muscle perimeter, defined as the outer border of the smooth muscle, and the perimeter of the basement membrane. From these measurements the total airway wall area was calculated as the difference between the adventitial border and the basement membrane. The airway wall was divided into the outer wall area (area between the adventitial border and smooth muscle) and the inner wall area (area between the smooth muscle and basement membrane).

When the four groups were compared (fatal and nonfatal asthmatics, COPD subjects and control subjects), the fatal asthmatic subjects demonstrated increased total airway wall thickness compared to those subjects with nonfatal asthma. This includes greater outer wall area, inner wall area, submucosal area and smooth muscle area. However, the airway walls in the nonfatal asthmatics were still 2–3 times thicker (greater outer wall, inner wall, submucosal and smooth muscle areas) than those subjects with COPD and control subjects. These data confirm those by JAMES et al. [64] where the submucosal area in asthmatics was also found to be increased. A study by CARROLL et al. [84] also revealed similar results, but they looked at several different sizes of airways, ranging <2 to >1.8 mm. Because of their study design, differences in inner wall area, outer wall area, smooth muscle area, mucous gland area and

Table 1. – Summary of study results quantifying airway structural elements of the large and small airways in asthma

First author [Ref.]	Areas evaluated	Population studied	Results
Studies supporting significant distal airway involvement in asthma			
Kuwano [83]	membranous bronchioles wall thickness	FA NFA mild COPD control subjects	total airway wall thickness: FA>NFA> COPD and control subjects
	blood vessels	same	vessels occupied a greater percentage of submucosa in FA number of vessels·mm^{-2} same in asthma, COPD and control subjects
Carroll [84]	large and small airways <2–18 mm inner and outer wall areas	FA NFA subjects	outer wall area: FA>NFA and control subjects in airways>10mm FA and NFA>control subjects in airways <2 mm inner wall area: FA>NFA in airways 10–18 mm FA and NFA >control subjects in airways <2 mm
Studies not supporting significant distal airway involvement in asthma			
Carroll [85]	blood vessels	same	vessels larger in FA but number·mm^{-2} same in both asthmatic and control groups when measurements combined; FA exhibited greater numbers and areas of large blood vessels and decreased numbers and area of small blood vessels in the 10–18 mm and >18 mm airways
James [64]	membranous and cartilaginous airways; epithelial, submucosal and smooth muscle areas	FA NFA control subjects	areas of epithelium, submucosa and muscle were greater in the asthmatic groups (combined) than the control subjects in all airways except in membranous bronchioles <2 mm.

COPD: chronic obstructive pulmonary disease; FA: fatal asthma; NFA: nonfatal asthma.

cartilage area between small and large airways were directly compared. The inner wall area, defined as the area encompassing the epithelial border to the smooth muscle, was similar between fatal and nonfatal asthmatics in the small airways (<2 mm and 2–4 mm) (fig. 5). The same observation held true for the outer wall area, defined as the area between the adventitial border and smooth muscle. As the airway size increased, the changes were more remarkable in the fatal asthma group. Thus, changes in the small airway structure are evident in both fatal and nonfatal asthmatics.

Blood vessel dimensions were also examined in two studies [83, 85]. Kuwano *et al.* [83] showed that both fatal and nonfatal asthmatic groups demonstrated similar blood vessel dimensions in the adventitia of membranous bronchioles, but the vessels of the fatal asthmatics took up more space in the submucosa as compared to the nonfatal asthmatics. However the number of vessels·mm^{-2} throughout the airway was the same in asthma, COPD and control subjects, suggesting that proliferation of vessels was appropriate for the increase in submucosal tissue.

Carroll *et al.* [85] also evaluated blood vessel size and distribution but in both large and small airways. They found that the number of blood vessels·mm^{-2} were similar in the fatal and nonfatal asthmatic and control groups when the measurements from all airways were combined. In patients with fatal asthma there were increased num-

bers and areas of large vessels and decreased numbers and areas of small blood vessels in segmental and lobar cartilaginous airways (10–18 mm and >18 mm) as compared to nonfatal asthmatics and control subjects. When the small airways were compared, small blood vessel and large blood vessel numbers and areas were similar between the three groups. The degree of dilatation of vessels was similar in each group and the number was ~80% of maximum. The significance of the increases in adventitial and submucosal areas, whether due to smooth muscle, cartilage, blood vessels or inflammatory cells, has been suggested by Macklem [86] to cause "uncoupling" of the airway smooth muscle from the surrounding parenchyma allowing it to shorten excessively.

Along these lines of discussion, the studies by Wiggs and coworkers [87, 88] incorporate the structural changes seen in the asthmatic airway into a model that may explain the alterations in the peripheral airways. They modelled a noncartilaginous conducting airway segment as a bilayered cylindrical structure. The model considered only the airway tissue internal to the smooth muscle layer. They demonstrated that the number of mucosal folds occurring during smooth muscle contraction is markedly increased in asthma, and the critical determinant of this pattern is the increased thickness of the inner layer represented by the enhanced subepithelial collagen deposition
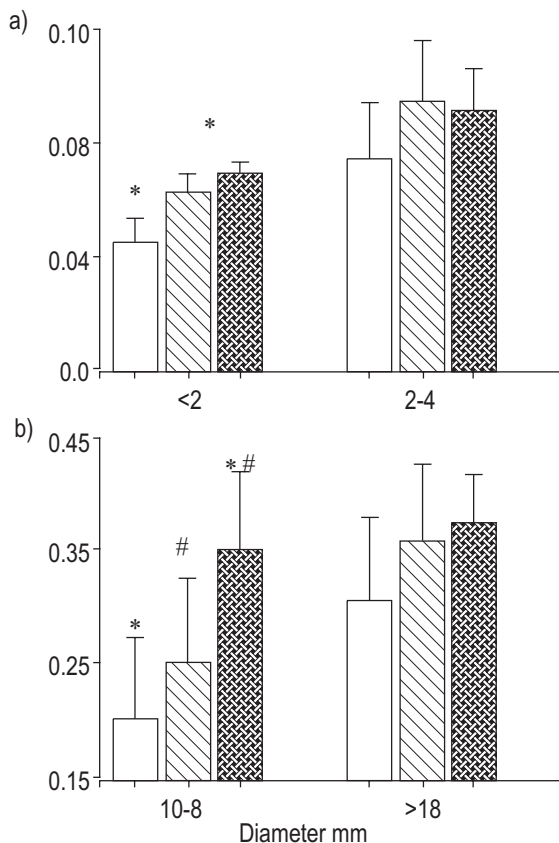
Fig. 5. – Inner wall area expressed as square millimetres per millimetre of basement membrane perimeter (Pbm) in large and small airways from cases of fatal asthma (▨), nonfatal asthma (▧), and control cases (□). Error bars represent one standard deviation. *,#: p<0.05, paired values. (Adapted from [84].)

and/or increased number of inflammatory cells. With these changes in the airway wall, the peripheral airways are very susceptible to significant obstruction and/or closure given the smaller internal diameter.

Airway inflammatory cells

Investigators have evaluated both cellular number and their products in the large and small airways both in fatal and nonfatal asthma [80, 81, 89–92]. There are studies that do and do not support significant distal airway inflammation in asthma. All of these studies are summarized in table 2. The studies not supporting a significant role for the distal airways in asthma come from autopsy and surgical studies [90, 91]. CARROLL *et al.* [90] evaluated the numbers of eosinophils, and lymphocytes in the large and small airways in fatal and nonfatal asthmatic subjects obtained *via* autopsy. There were 10 subjects in each group, and smoking status was not known. Cell counts were performed in the membranous and larger airways, and expressed as cells·mm⁻¹ of the basement membrane perimeter. The cells of interest were counted within a given area to obtain the number of cells·mm⁻², then multiplied by the area to obtain the total number of cells. This number was then divided by the basement membrane perimeter to obtain the number of cells·mm⁻¹ of the basement membrane perimeter. Of the three sizes of airways evaluated (<6 mm, 6–16 mm and >16 mm), the

numbers of lymphocytes·mm⁻¹ of the basement membrane perimeter was greatest in the nonfatal asthma group for the <6 mm and greater in the fatal and nonfatal asthma groups as compared to controls in the 6–16 mm airways, and greatest in the fatal asthma group in the large (>16 mm) airways examined. The number of eosinophils·mm⁻¹ of basement membrane perimeter was significantly greater in all the airways of the fatal asthmatic group. To evaluate these data critically, it must be remembered that the total length of basement membrane is significantly greater in the peripheral airways than the central airways due to the increased cross sectional area. Although the number of cells per unit basement membrane may be low, the total number of cells in the airway wall is much greater in the peripheral lung.

FAUL *et al.* [91] evaluated the airway histology from five asthmatics dying within 1 h after the onset of symptoms. Using the monoclonal antibodies to identify T-cell subsets (CD4 and CD8), eosinophils (EG2) and macrophage subtypes (D1, D7 and D1/D7) expressed in number·μm⁻², they revealed a mononuclear infiltration throughout the large and small airways composed of greater numbers of CD8+ than CD4+ cells, normal proportions of macrophages and a significant number of activated eosinophils compared to historical asthma control subjects. Some comparisons between proximal and distal airways were performed, with greater numbers of macrophages and eosinophils appreciated in the proximal as compared to distal tissue. Similar to the study by CARROLL *et al.* [90] the numbers of T-cells were also greater in the proximal airways, but did not reach statistical significance.

In support of significant distal airway inflammation, HAMID *et al.* [92] evaluated inflammation in the large and small airways from asthmatics and nonasthmatics who underwent thoracic surgery for treatment of pulmonary carcinoma. Six subjects were studied in the asthma group and 10 in the nonasthma group. The mean cigarette use in pack-years was 40.8±31.9 and 41.9±26.8, respectively. Airways > and <2 mm in diameter were evaluated for T-cells, total eosinophils using a major basic protein antibody, activated eosinophils using EG2 antibody, mast cells using the antibody directed against tryptase (AA1), neutrophils using anti-neutrophil elastase and macrophages using the CD68 antibody.

In airways <2 mm in diameter, the numbers of T-cells, total eosinophils and activated eosinophils were greater in asthmatics as compared to control subjects. The same results were shown for airways >2 mm in diameter in addition to increased mast cells (fig. 6). In contrast to the autopsy studies, when the large and small airways were compared directly, there were greater numbers of activated (EG2+) eosinophils in the small airways as compared to the larger airways (fig. 6). When the subjects using inhaled corticosteroids were excluded and the data reanalysed, the results were still significant. These findings suggest that patients with chronic asthma may exhibit significant distal airway inflammation regardless of inhaled corticosteroid use.

Further studies by KRAFT *et al.* [80, 82] and WENZEL *et al.* [81] in moderate asthmatics, and severe asthmatics, respectively support these studies. However, the lung parenchyma and large airways were evaluated. KRAFT *et al.* [80, 82] performed transbronchial and endobronchial biopsies in subjects with and without significant nocturnal worsening of asthma at 16:00 h and 04:00 h. The

Table 2. – Summary of study results quantifying inflammatory cells of the large and small airways in asthma

First author [Ref.]	Areas evaluated	Population studied	Results
Studies supporting significant distal airway involvement in asthma			
KRAFT [82]	large airways *via* endobronchial biopsy and alveolar tissue *via* transbronchial biopsy	asthmatics with nocturnal worsening of lung function; (NA) asthmatics without worsening of lung function (NNA)	increased alveolar tissue CD4+ cells in NA as compared to NNA at 04:00 h; significant inverse correlation between alveolar tissue EG2+ and CD4+ cells and FEV1; significant positive correlation between alveolar tissue CD4+ and EG2+ cells
HAMID [92]	airways >2 mm and <2 mm	NFA and control subjects undergoing thoracic surgery	increased CD3+, EG2+ MBP+ and AA1+ cells in air asthmatics compared to control subjects in airways >2 mm; increased CD3+, EG2+ and MBP+ cells in asthmatics compared to control subjects in airways <2 mm; increased EG2+ cells in airways <2 mm as compared to airways >2 mm
WENZEL [81]	large airways *via* endobronchial biopsy and alveolar tissue *via* transbronchial biopsy	severe and moderate asthmatics (tissue comparison); controls included in BAL comparison only	increased neutrophils in the large airways and alveolar tissue of severe asthmatics as compared to moderate asthmatics
KRAFT [80]	large airways *via* endobronchial biopsy and alveolar tissue *via* transbronchial biopsy at 16:00 h and 04:00 h	asthmatics with nocturnal worsening of lung function; (NA) asthmatics without nocturnal worsening of lung function (NNA)	increased alveolar tissue eosinophils in NA as compared to NNA at 04:00 h; increased eosinophils in alveolar tissue within NA group at 04:00 h as compared to 16:00 h
Studies not supporting significant distal airway involvement in asthma			
CARROLL [90]	large and small airways <6 mm, 6–16 mm and >16 mm number per basement membrane perimeter	FA NFA control subjects	lymphocytes: FA>NFA and controls in >16 mm airways FA and NFA > control subjects in airways <6–16 mm; NFA>FA and control subjects in airways <6 mm. eosinophils: FA>NFA and control subjects in all airways sampled
FAUL [91]	proximal airway >1 mm distal airway <1 mm plus alveolar tissue	FA	increased CD8+ cells and macrophages in proximal *versus* distal tissue, but not statistically significant

NA: nocturnal asthma; NNA: non-nocturnal asthma; EG2+: eosinophil subtype EG2 positive; NFA: nonfatal asthma; MBP+: major basic protein positive; AA1: the antibody directed against tryptase; BAL: bronchoalveolar lavage; FA: fatal asthma.

asthmatics with nocturnal worsening of symptoms, hereby referred to as nocturnal asthma, demonstrated increased numbers of eosinophils in the lung parenchyma as compared to the asthmatics without nocturnal worsening at 04:00 h (fig. 7). Additionally, the numbers of eosinophils were greater in the lung parenchyma per unit volume at 04:00 h as compared to 16:00 h (fig. 7). The number of alveolar, not endobronchial tissue eosinophils inversely correlated with the overnight fall in FEV1 (r=-0.54, p=0.03). In a follow-up study with a similar design, monoclonal antibodies were used to determine the number·mm^{-2} of T-cell subsets (CD3, CD4 and CD8) and EG2+ eosinophils in the proximal endobronchial and distal transbronchial (alveolar) tissue [82]. At 04:00 h, the nocturnal asthma group had significantly greater CD4+ cells in the alveolar tissue per unit area as compared to the non-nocturnal asthma group (9.8 cells·mm^{-2} [5.6–30.8,

interquartile range (IQ)) *versus* 1.5 cells·mm^{-2} (0–6.3, IQ), p=0.04). Within the nocturnal asthma group, there were significantly greater numbers of CD3+, CD4+, CD8+ and EG2+ cells in the endobronchial lamina propria (~0.15 mm below the basement membrane) as compared to the alveolar tissue at both 16:00 h and 04:00 h. There were no differences in numbers of inflammatory cells within the epithelial compartment between the groups at either time point. However, only alveolar tissue, not airway tissue CD4+ cells, correlated inversely with the percentage predicted FEV1 at 04:00 h (r=-0.68, p= 0.0018) and positively with the number of alveolar tissue EG2+ cells (r=0.75, p=0.002), suggesting that the relative increase in CD4+ lymphocytes in the alveolar tissue at night in nocturnal asthma as compared to non-nocturnal asthma could be responsible for orchestrating eosinophil influx and activation in nocturnal asthma.
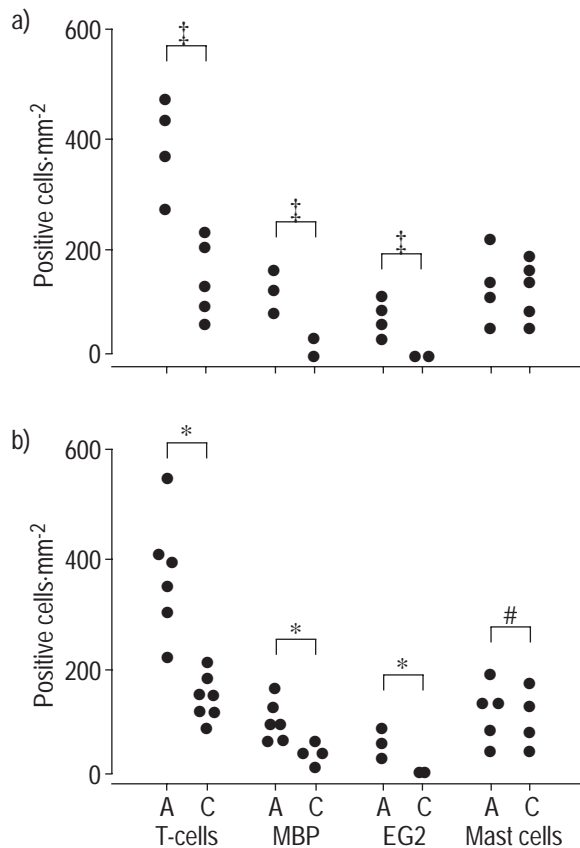
a)

b)

Fig. 6. – Immunocytochemical cell markers in airways a) <2 mm in diameter and b) airways >2 mm in diameter, from patients with asthma (A) and control subjects (C). T-cells (CD3), total eosinophils (MBP), activated eosinophils (EG2), and mast cells (tryptase) were studied. Values given are average data of six (<2 mm) or 10 (>2 mm) airways from four to six patients with asthma and 13 (<2 mm) or 31 (>2 mm) airways from six to 10 nonasthmatic control subjects. ‡: p<0.02; *: p<0.001; $^{\#}$: p<0.005. (Adapted from [82].)

Employing endobronchial and transbronchial biopsy in severe asthma, WENZEL et al. [81] revealed that neutrophils were present and increased in the proximal endobronchial tissue, the distal alveolar tissue and BAL of severe, steroid dependent asthmatics as compared to moderate asthmatics and control subjects. All subjects demonstrated ≥15% improvement in FEV1 with an inhaled β2-agonist. Although somewhat controversial, the summary of the data suggests that inflammation is present in asthma from the large airways to the lung parenchyma.

Transbronchial biopsy is not a risk free procedure secondary to the risk of pneumothorax. Therefore, assessment of distal inflammation in humans has been performed through the use of BAL employing a double-lumen catheter technique [93] or through evaluation of initial and subsequent BAL aliquots [94–99]. With the latter technique, it is not entirely clear where in the airway the sample originated, but it can shed light on the presence of distal inflammation in asthma. VAN VYVE et al. [94] evaluated this technique by naming the first 50 mL aliquot of saline the "bronchial sample" and the subsequent four 50 mL aliquots the "alveolar sample" in control and asthmatic subjects. They noted that the alveolar sample contained significantly more cells than the bronchial sample. Within the asthmatic group, the bronchial sample contained sig-

nificantly greater percentages of neutrophils, eosinophils and epithelial cells, while the alveolar sample contained significantly greater percentages of macrophages and lymphocytes. Using the scoring system of Aas [100], the investigators reported a significant correlation between alveolar, not bronchial eosinophils, and the score of Aas [100]. Although weak, it was stronger than that seen with the bronchial sample eosinophils (r=0.25, p=0.024 and r= 0.38, p=0.0006 for bronchial and alveolar eosinophils, respectively). These studies can be useful, but the precise compartment sampled may vary from patient to patient.

Imaging studies to evaluate the distal lung

Imaging the lung to detect air trapping and airway closure has been used as a non-invasive marker of small airways function in asthma [101–109]. One method is via high resolution computed tomography (HRCT) scan. Several studies have been published in this area [101–106]. NEWMAN et al. [101] used the pixel index, in which the percentage of pixels <-900 Hounsfeld units is used to assess the degree of air trapping in asthmatics at end expiration. As HRCT density of normal lung is -700– -800 on inspiration, the pixel index describes areas of low attenuation thought to be consistent with air trapping. These investigators found that the pixel index at a level immediately superior to the diaphragm was significantly higher in asthmatics as compared to control subjects, and was therefore consistent with increased air trapping. This index positively correlated with the residual volume (r= 0.63, p<0.006) and negatively with the FEV1 (r=-0.65, p<0.004) and forced vital capacity (FVC) (r=-0.58, p<0.02).

These data were confirmed by CARR et al. [104], where patients with severe asthma underwent HRCT scanning to determine air trapping. Instead of using the pixel index, they quantified the area where air trapping was present. They defined air trapping as decreased attenuation and failure to reduce in volume with an associated increase in pulmonary vascular markings. The severe asthmatics (FEV1 56.6±4.1% predicted) exhibited a significantly greater area of air trapping as compared to historical nonasthma control subjects (75.9±2% versus 44.6±1.0%) and a negative correlation with the FEV1 (r=-0.60, p<0.001). Despite the limitation of the use of historical controls, this study supports the hypothesis that air trapping by HRCT is increased in asthma and correlated with lung function.

KING and coworkers [107, 108] expanded these observations by employing single photon emission computed tomography (SPECT) and inhaled Technegas, an ultrafine aerosol of carbon particles labelled with ^{99m}Tc to investigate airway closure. SPECT scanning offers the advantage of a three dimensional image, thus potentially avoiding any overlap in structures which can occur with HRCT. These investigators defined airway closure as the percentage of Technegas free lung volume. They compared this value to closing volume measured via a modification of the single breath nitrogen washout test described by BUIST et al. [110]. Closing capacity was defined as the sum of the closing volume and residual volume. In asthmatics, the distribution of airway closure was predominately basal, but only correlated with closing capacity, but only in control subjects (r=0.86, p<0.01). In asthmatic subjects, airway closure was measured by the SPECT/
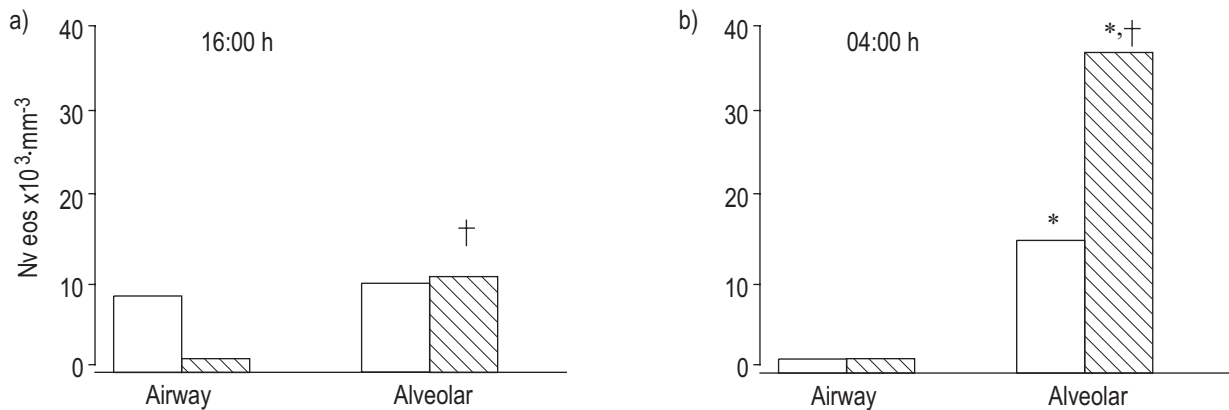
Fig. 7. – The median number per volume (Nv) of eosinophils (eos) in the nocturnal asthma (NA; ▨) and non-nocturnal asthma (NNA; □) groups are shown in the endobronchial (EB; airway tissue) and transbronchial (TB; alveolar tissue) biopsies. a) EB, NNA 25–75 interquartile range (IQ): 1.9–18.6; EB, NA IQ: 0.0–13.3; TB, NNA IQ: 18.2–29.1; TB, NA IQ: 2.7–16.8. b) EB, NNA IQ: 0.0–0.0; EB, NA IQ: 0.0–1.8; TB, NNA IQ: 3.0–26.5; TB, NA IQ: 17.7–75.0. *, †: p≤0.05. (Adapted from [80].)

Technegas method and did not correlate with physiological variables. Therefore, closure of the airways in asthmatic subjects was not occurring in the same way as that in control subjects. The distribution was more patchy and uneven, with discrete, wedge-shaped defects observed in some, suggesting large airway closure in some subjects. Closure of the small airways, as described above, may result from mucus plugging, increased smooth muscle tone or from structural changes such as those modelled by WIGGS et al. [88], which can result in excessive airway closure and instability. In addition, the lack of correlation with closing capacity may be secondary to underestimation of the closing volume by the single breath nitrogen washout technique [111, 112].

TASHKIN et al. [109], using radioxenon, were able to determine the effect of aerosolized and subcutaneously administered terbutaline on xenon washout and expiratory flow. In 12 asthmatic subjects, specific airway conductance (sGaw), peak expiratory flow and the ratio of maximal expiratory flow at 50% of vital capacity breathing 80% helium, 20% oxygen to that breathing air (ratio V'max50) were determined before and after the administration of aerosolized terbutaline (0.5 mg), subcutaneous terbutaline (0.5 mg) or placebo. Increases in sGaw and peak expiratory flow of ≥25% were hypothesized to indicate significant dilatation of central airways; increases in ratio V'max50 of ≥0.10 was hypothesized to reflect dilatation of peripheral airways. In addition, radioaerosol and radioxenon lung imaging was performed to determine the relationship between changes in lung imaging patterns and changes in physiological indices in response to bronchodilator therapy. There was no significant change in lung function or radioxenon images after placebo. After inhaled terbutaline, the results were variable: sGaw and peak expiratory flow increased ≥25% in 7/12 subjects, ratio V'max50 increased ≥0.10 in only three subjects, radioaerosol images showed less central deposition in nine subjects and radioxenon images showed improved distribution and/or washout of xenon in five subjects. After the administration of subcutaneous terbutaline, sGaw and peak expiratory flow increased ≥25% in 10 subjects, ratio V'max50 increased ≥0.10 in 10 subjects, and radioaerosol and xenon images showed improvement in 11 and eight subjects, respectively. These findings are consistent with the action of inhaled terbutaline mainly on large airways and of subcutaneous terbutaline on both large and small airways. Although reduced central radioaerosol deposition correlated well with physiological evidence of large airway dilatation, improvement in xenon distribution and washout could be attributed to dilatation of either large and/or small airways.

The distal lung in asthma: is it important?

The finding of distal airway inflammation in asthma is significant, as it is currently not clear if inhaled corticosteroids, the mainstay of asthma therapy, effectively treat this compartment of the lung. It has been shown that treatment, with inhaled corticosteroids does not normalize hyperresponsiveness [113]. In addition, a study by ESMAIL-POUR et al. [114] investigated the deposition pattern of fluticasone propionate in peripheral and central lung tissue obtained from asthmatics undergoing thoracic surgery. Their study revealed significantly greater tissue concentration of fluticasone in the central tissue as compared to the peripheral tissue. One potential method to address this issue is by altering the propellants, and hence the particle size which may alter medication delivery. LEACH et al. [115] have shown that beclomethasone in hydrofluoroalkane (HFA) propellant reached the peripheral airways more effectively than beclomethasone in chlorinated fluorocarbon propellant (CFC). These data must be interpreted with caution, as labelling particles for visualization can alter the distribution properties.

Evaluation of physiological and pathological studies suggest that the distal lung units, including the small airways (<2 mm) and lung parenchyma, participate in the pathogenesis of asthma. However, the challenge lies in improving the ability to assess their structure and function noninvasively. Physiological studies of lung hysteresis reveal important relationships between the airways and parenchyma, and potentially provide a way to assess which compartment of the lung is contributing the most significantly in a given asthmatic. However, the precise site of obstruction is still controversial in these studies. Bronchoscopic measurements of peripheral resistance offer a direct but still invasive method to evaluate this compartment of the lung. Imaging studies are particularly exciting, as they

are noninvasive. Before these techniques can be widely used, they must be validated through pathological and physiological techniques such as biopsy and bronchoscopic peripheral resistance measurements. Although challenging, the endeavour to evaluate the distal lung is definitely worth the effort, as several studies have illustrated that if the distal lung is participating significantly in asthma pathogenesis in a given patient, there, are clinical implications [60, 61]. Therefore, the distal lung in asthma is anything but quiet.

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