



Appraising the small airways in asthma

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Purpose of review

The small airways play an important yet poorly targeted role in asthma pathophysiology, leading to increased morbidity in asthma patients. Assessing inflammation and remodeling in these airways, determining the contribution of small airways to lung dysfunction and enhancing drug delivery to the distal regions of the lung remain challenging. The purpose of this review is to highlight recent advances in our understanding of small airways involvement in asthma.

Recent findings

Inflammation in the small airways can be evaluated through exhaled gas measurements, most often nitric oxide. However, additional exhaled biomarkers have recently been described. Considerable infiltration of mast cells in the distal lung and extensive structural changes to the small airways have also been demonstrated. Advances have been made in the functional assessment of small airways, particularly in the measurement of small airway compliance and ventilation defects and in studies investigating the impact of small particle inhaled corticosteroid treatment on lung function.

Summary

Experimental assessments of small airways inflammation, remodeling and function have provided novel insights into the importance of the distal regions of the lung in asthma pathology. Further advances in drug delivery to the small airways have the potential to improve asthma control.

Keywords

asthma, inflammation, pharmacotherapy, physiology, small airways

INTRODUCTION

The pathophysiology of asthma involves inflammatory and structural changes that have, for the most part, been described in the large airways. Due to technical considerations, it has been considerably easier to sample the large airways in human asthmatic patients, for example by induced sputum to assess inflammation and endobronchial biopsy to evaluate structural remodeling. However, a number of studies investigating lung tissue from autopsy and surgical resections have indicated that eosinophilic inflammation also occurs, often to a greater degree, in the small airways (<2 mm lumen diameter) of asthmatic patients [1]. The small airways have also been shown to express increased levels of a number of key cytokines and chemokines in the distal lung, including interleukin (IL)-4, IL-5, eotaxin and monocyte chemoattractant protein-4 [1]. In terms of lung function, the idea that the distal lung is a 'quiet zone', which is responsible for only 10% of airway resistance, has been challenged by recent advances in the assessment of small airway resistance by frequency-dependent techniques [1]. Taken together, our current understanding of small airway biology supports

an important, although poorly targeted, role of the distal airways in asthma pathology.

Recent advances in biopsy methods, improvements in lung function measurements and novel imaging modalities have renewed interest in the distal lung in asthma and provided better insight into the role of the small airways in asthma. Considerable effort has gone into assessing small airway inflammation noninvasively by evaluating exhaled biomarkers. Invasive techniques have also been further developed, such as transbronchial biopsy, which can be used to monitor both inflammation and remodeling in the small airways of asthmatic patients. Advances have also been made in the measurement of functional parameters of the small

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KEY POINTS

- Assessing inflammation, remodeling and dysfunction in the small airways remains challenging.
- Recent studies have validated the use of a wide range of exhaled biomarkers to measure inflammation in the small airways.
- Novel methods are available to assess functional abnormalities of the small airways.
- Advances in drug delivery to the small airways have the potential to improve asthma control.

airways, including small airway resistance, compliance and ventilation, and in the enhancement of drug delivery to this compartment of the lung.

ASSESSING INFLAMMATION OF THE SMALL AIRWAYS

Noninvasive techniques to measure the composition of exhaled gases and breath condensates are established clinical methods for assessing small airway inflammation and oxidative stress in a number of lung diseases. Most often, these techniques measure fractional exhaled nitric oxide (FeNO), which has been validated as a marker of eosinophilic inflammation. This assessment is important because it has been shown that a sputum eosinophil count greater than 3% is responsive to corticosteroid treatment, whereas noneosinophilic asthma (<3% eosinophils) is less responsive to this intervention [2]. This clinically relevant threshold level of sputum eosinophilia has recently been associated with a threshold FeNO value of 42 ppb, which can be used to discriminate between eosinophilic and noneosinophilic asthma and to assess the efficacy of anti-inflammatory treatment in a well tolerated, rapid and noninvasive manner [3^{''}]. Further refinements have been made to this technique to assess the central and peripheral contributions to total lung FeNO levels using a ventilation heterogeneity approach [4], whereas Williamson *et al.* [5] have proposed a novel method to correct for nitric oxide production in the conducting airways, which allows for more accurate assessment of alveolar nitric oxide production and eosinophilic inflammation. In addition, several novel exhaled biomarkers for distal lung inflammation have recently been evaluated, including endothelin-1 [6], eotaxin-1 [7], RANTES [8] and hydrogen peroxide [9], all of which have been positively associated with inflammation. Interestingly, Sethi *et al.* [10] have found that levels of exhaled nitric oxide

were modulated by the type of bronchoprovocation test used (methacholine vs. allergen), further demonstrating that an inflammatory stimulus, that is allergen challenge, has a direct positive effect on nitric oxide levels.

The degree and character of inflammation in the small airways can be directly compared with inflammation in the large conducting airways using invasive techniques such as endobronchial biopsies (containing the bronchial wall and some peribronchial structures) and transbronchial biopsies (including a sample of the lung parenchyma) on the same patient [11]. This approach has been used recently by a number of investigators to assess the distribution and phenotype of inflammatory cells, particularly mast cells, in the large and small airways of asthmatic patients. These studies have found that asthmatic patients have more mast cells in the distal lung compared with the large airways [12^{''},13]. These regional differences in mast cell numbers were not seen in healthy controls, and the mast cells of asthmatic patients expressed higher levels of the high-affinity IgE-receptor FcεRI and increased surface-bound IgE [12^{''}]. These authors attributed the reduced density of mast cells in the large conducting airways to treatment with corticosteroids because this treatment is known to reduce mast cell numbers in the central airways [14]. It can be concluded from these studies that IgE-mediated inflammatory reactions are not limited to the large airways, but are also an important component of distal allergic lung disease. Therefore, pharmacological targeting of peripheral airway inflammation in asthma patients may provide a significant clinical benefit.

SMALL AIRWAY REMODELING

In patients with fatal asthma, extracellular matrix (ECM) changes have been found to occur throughout the lung. In fatal asthma, these ECM changes are particularly intense in the outer region of the wall of small airways (Fig. 1a), including increased deposition of fibronectin, collagen I and collagen III, as well as increased expression of several metalloproteinases (MMP-1, MMP-2 and MMP-9) [15]. Changes in these regions are associated with perturbed alveolar attachments and may contribute to airway-parenchyma uncoupling and altered mechanics of the distal lung.

Regional variability in the phenotype of lung structural cells has been demonstrated recently in a study by Pechkovsky *et al.* [16^{''}] in which normal primary fibroblasts from the proximal and distal airways were assessed for their responses to transforming growth factor (TGF)-β. It was found that

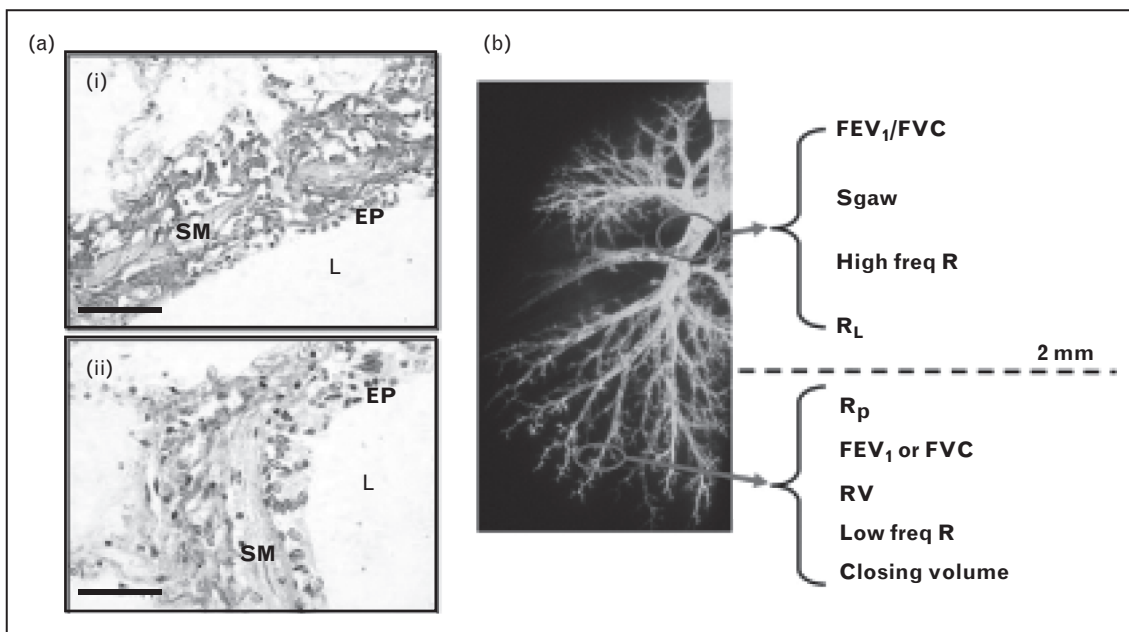


FIGURE 1. Anatomy, functional parameters and histology of the small airways. (a) Immunohistochemical staining for collagen I in the small airways of an asthmatic patient (i) and a healthy individual (ii). EP, epithelium; L, lumen; SM, smooth muscle. Scale bars 50 μm. Reproduced with permission from Dolnikoff *et al.* [15]. (b) Anatomy of the tracheobronchial tree and the associated physiological lung function measurements in the proximal and distal airways. FEV₁, forced expiratory volume in 1 s; freq R, resistance at low or high frequencies; FVC, forced vital capacity; R_L, lung resistance; R_p, peripheral resistance; S_{gaw}, specific airway conductance. Reproduced with permission from Hyde *et al.* [1].

distal fibroblasts showed evidence of enhanced baseline TGF-β signaling and showed more of a myofibroblast phenotype with higher baseline expression of α-smooth muscle actin. Distal fibroblasts were also shown to be stiffer and more contractile compared with cells from proximal regions of the lung. The results of this study demonstrate that mesenchymal cells of the distal lung have an enhanced ability to differentiate into myofibroblasts under conditions of increased TGF-β expression and mechanical tension. However, the contributions of these cells to distal lung fibrosis, as well as regional phenotypic differences in mesenchymal cell populations in asthmatic patients, are yet to be investigated.

FUNCTIONAL ASSESSMENTS OF THE SMALL AIRWAYS

Abnormal airway function may result from altered smooth muscle dynamics, airway remodeling of the large and small airways, and a loss of parenchymal tethering to the airways. Methods used to evaluate lung function must therefore have the capacity to not only provide data on resistance in the large airways but also resistance in the small airways along with other measures of lung function such as airway compliance.

Respiratory airflow measurements using forced expiratory flow (spirometry, forced expiratory volume in 1 s) provide useful information on obstruction of the large conducting airways, but are less useful for assessing impaired function of the small airways (Fig. 1b). Moreover, there is often a poor correlation between spirometry measurements and asthma symptoms or airway inflammation, especially in asthmatic children [17,18]. Alternative measurements have therefore been established to assess small airway impairment, such as impulse oscillometry, which uses repetitive pressure pulses at the mouth to calculate small airway resistance [19]. This method uses derivative models of the well known Mead model to eliminate upper airway artifacts and provide data only on the distal lung compartment. This method was used with variable success by Goldman *et al.* because the data were less reliable in patients with severe obstruction. A recent study by Keen *et al.* [20] investigated the correlation between measures of airway dysfunction (LCI, the lung clearance index; S_{cond}, the contribution of the conducting airway zone; and S_{acin}, the contribution of the acinar lung zone) and noninvasive measurements of small airway inflammation (FeNO) in pediatric healthy and asthmatic individuals. A significant correlation was found between the indices for small airway resistance and small airway inflammation.

Dynamic lung compliance is defined as the distensibility or stretch capacity of the lung at a particular pressure. Fibrosis, often associated with severe asthma, results in a loss of lung compliance and increased stiffness, whereas emphysematous changes lead to increased lung compliance due to a loss of elastic recoil. Airway compliance has been persistently difficult to measure and quantify in humans. A recent publication by Kelly *et al.* [21[¶]], however, describes a novel method for compliance measurements using a curve-fitting algorithm combining pressure–volume curves with measurements of airway diameter from high-resolution computed tomography scans. This method creates diameter–pressure curves for airways of variable size, from which specific airway compliance can be determined at any transpulmonary pressure. This method was applied to data from two healthy individuals, and showed greater compliance in the small airways compared with the large airways. Although further investigation and validation are required, this technique may be useful for determining the degree of fibrosis and airway stiffness in asthmatic patients using non-invasive data collection techniques.

VENTILATION IMAGING

Noninvasive imaging employing MRI with hyperpolarized noble gases is a novel method to investigate regional variations in ventilation in chronic lung disease. Hyperpolarized ^3He and ^{129}Xe are inert, non-toxic, inhaled contrast agents that can be detected by MRI to provide a high-resolution image of ventilated lung airspaces. Advantageously, this technique uses no ionizing radiation, allowing for prolonged and repeated assessments over time in the same patient. Previously, ^3He was the preferred contrast agent for these assessments, because higher polarization rates, and therefore a better signal, could be achieved. However, recent advances in polarizer technology have improved the quality of images using ^{129}Xe , and ^{129}Xe can moreover be used to evaluate gas exchange from the air spaces into the tissue/blood, which cannot be achieved with ^3He [22[¶]].

In healthy individuals with normal lung function, inhaled gases are distributed rapidly and evenly throughout the lung, which results in a uniform signal. In patients with obstructive lung diseases, including chronic obstructive pulmonary disease (COPD), cystic fibrosis and asthma, focal ventilation defects appear as dark areas on MRI. The disorder behind these ventilation defects could be associated with mucus plugging, structural defects or airway narrowing. Ventilation defects are common in asthma, and the degree of these defects has been positively correlated with disease

severity [23]. Recent advances have demonstrated that constriction of the small airways is required for ventilation defects. By synthesizing ^3He MRI data with measures of lung function, Campana *et al.* [24] generated ventilation images from healthy and asthmatic individuals to spatially map locations of defective ventilation in the lung; this technique could be used to visualize ventilated and nonventilated airways under baseline conditions, after methacholine challenge and following albuterol administration. The functional lung imaging data obtained in this study showed considerable heterogeneity in poorly ventilated areas of the distal lung in asthmatic patients, and further demonstrated that only a 70% construction of the small airways could generate the ventilation defects observed by ^3He MRI.

More recently, Tustison *et al.* used MRI with ^3He as the contrast agent to validate this technique as an alternative measure of lung function. In this study, ^3He MRI assessments were made before and after respiratory challenge, and the results were compared in a computational framework with data obtained through classical spirometry in healthy ($n=8$) and asthmatic ($n=47$) individuals. Taken globally, the features of ventilation imaging were better than spirometry in establishing the clinical diagnosis of these individuals. Specifically, the information acquired by postchallenge ^3He MRI provided an excellent appraisal of regional lung ventilation with good discriminatory power between healthy and asthmatic individuals [25[¶]]. However, this technique requires further validation in patients with severe asthma because large areas of severe ventilation defects impair correct processing of the imaging data.

Mugler *et al.* [22[¶]] recently published a report on the simultaneous, rapid ($<20\text{s}$) imaging of lung ventilation and gas exchange employing ^{129}Xe as the MRI contrast agent. By exploiting the ratio of the differential MRI signal for ‘gas-phase’ to ‘dissolved-phase’ ^{129}Xe , these investigators were able to assess gas exchange in healthy, asthmatic and mild COPD individuals in different areas of the lung. Gas exchange in healthy individuals was robust, whereas individuals with chronic lung disease showed impaired gas uptake, but not necessarily in areas in which ventilation defects were present. These results suggest that gas exchange in the lung is not only dependent on ventilation but also on some tissue-specific aspects such as structural remodeling and local blood flow.

DRUG DELIVERY TO THE DISTAL LUNG

Inhaled corticosteroids are the gold standard in the treatment of allergic asthma. However, typical

corticosteroid formulations (e.g. fluticasone, beclomethasone and budesonide) are delivered as relatively large particles ($>3\mu\text{m}$) that do not adequately penetrate into the distal regions of the lung. Early studies investigating small particle formulations of inhaled corticosteroids (e.g. QVAR, a hydrofluoroalkane-driven beclomethasone extra-fine inhalation aerosol) demonstrated improved deposition of these drugs in the distal lung and efficacy equivalent to inhaled budesonide at half the dose [26]. More recently, ciclesonide, a novel corticosteroid with similar efficacy to fluticasone delivered as small particles ($1.5\text{--}3\mu\text{m}$), has been shown to penetrate into the peripheral regions of the lung [27]. A recent study in 30 mild persistent asthmatic patients demonstrated the positive effects of this corticosteroid formulation on small airway resistance in a side-by-side comparison with fluticasone. Treatment with ciclesonide for a period of 8 weeks resulted in a significant improvement, compared with fluticasone treatment, in small airway function assessed by impulse oscillometry [28^{***}]. Similar results were found in a study in which mild asthmatic patients were treated either with an ultra-fine formulation of beclomethasone/formoterol or a standard formulation of fluticasone propionate/salmeterol. Improvements in some aspects of small airway function were seen in the group of patients given the ultrafine formulation, although the two showed an equivalent improvement in measures of large airway function [29^{*}].

Concerns have arisen regarding the comparative safety of small particle formulations. Some investigators have proposed an improved safety profile for small particle drugs as a result of reduced drug deposition in the oropharynx associated with a decreased incidence of oropharyngeal candidiasis. However, other studies have suggested that small particle formulations of ciclesonide are associated with impaired growth in children as well as hypothalamic–pituitary–adrenal axis suppression in adults, although these events may be related to the ability of ciclesonide to bind to serum proteins rather than to the size and deposition of the particles in the distal lung [27].

Beyond treatment with corticosteroids, montelukast, a systemically bioavailable leukotriene receptor antagonist, has been shown to improve functional measures of distal lung disease in asthmatic patients [30]. Moreover, montelukast significantly reduces alveolar FeNO production when given concurrently with inhaled fluticasone, but not when given alone [31]. These results suggest that co-administration of these drugs has effects on small airway inflammation that cannot be adequately addressed with monotherapy, thus

supporting an earlier study evaluating global nitric oxide production [32]. With these encouraging results, more research is certainly warranted to further elucidate the impact of montelukast therapy on inflammation and remodeling of the distal lung in asthma.

CONCLUSION

The impact of inflammation and remodeling in the small airways has received increased attention in recent years. Although a number of new techniques to assess the pathology of the distal lung have been established, further validation in larger groups of asthmatic patients of varying disease severity is warranted. Further studies are needed to gain a clearer picture of the small airways in asthma and to define a treatment strategy that adequately addresses distal lung disease.

Conflicts of interest

J.R.J. has no conflicts of interest to disclose. Q.H. has served on the advisory board of Geisi Italy.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 91).

1. Hyde DM, Hamid Q, Irvin CG. Anatomy, pathology, and physiology of the tracheobronchial tree: emphasis on the distal airways. *J Allergy Clin Immunol* 2009; 124:S72–S77.
2. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Noneosinophilic corticosteroid unresponsive asthma. *Lancet* 1999; 353:2213–2214.
3. Schleich FN, Seidel L, Sele J, *et al.* Exhaled nitric oxide thresholds associated with a sputum eosinophil count $>1 = 3\%$ in a cohort of unselected patients with asthma. *Thorax* 2010; 65:1039–1044.
- This study evaluated the relationship between sputum eosinophil counts and exhaled nitric oxide in 295 asthmatic individuals; a FeNO threshold of 42 ppb was found to discriminate between eosinophilic and noneosinophilic asthma with a cut-off value of at least 3% eosinophils.
4. Verbanck S, Schuermans D, Vincken W. Inflammation and airway function in the lung periphery of patients with stable asthma. *J Allergy Clin Immunol* 2010; 125:611–616.
5. Williamson PA, Clearie K, Menzies D, *et al.* Assessment of small-airways disease using alveolar nitric oxide and impulse oscillometry in asthma and COPD. *Lung* 2011; 189:121–129.
6. Zietkowski Z, Skiepkowski R, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A. Anti-IgE therapy with omalizumab decreases endothelin-1 in exhaled breath condensate of patients with severe persistent allergic asthma. *Respiration* 2010; 80:534–542.
7. Zietkowski Z, Tomasiak-Lozowska MM, Skiepkowski R, *et al.* Eotaxin-1 in exhaled breath condensate of stable and unstable asthma patients. *Respir Res* 2010; 11:110.
8. Zietkowski Z, Skiepkowski R, Tomasiak-Lozowska MM, *et al.* RANTES in exhaled breath condensate of patients with severe persistent allergic asthma during omalizumab therapy. *Int Arch Allergy Immunol* 2011; 154:25–32.
9. Teng Y, Sun P, Zhang J, *et al.* Hydrogen peroxide in exhaled breath condensate in asthma: a promising biomarker? *Chest* 2011; 140:108–116.
10. Sethi JM, White AM, Patel SA, *et al.* Bronchoprovocation testing in asthma: effect on exhaled monoxides. *J Breath Res* 2010; 4:047104.
11. Boulet LP. Transbronchial biopsy as a tool to evaluate small-airways disease in asthma. *Pros Eur Respir J* 2002; 20:247–248.

12. Andersson CK, Bergqvist A, Mori M, *et al.* Mast cell-associated alveolar inflammation in patients with atopic uncontrolled asthma. *J Allergy Clin Immunol* 2011; 127:905–912.
This small study found that patients with atopic uncontrolled asthma have increased parenchymal infiltration of mast cells compared with atopic and nonatopic controls.
13. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005; 171:431–439.
14. Bentley AM, Hamid Q, Robinson DS, *et al.* Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996; 153:551–556.
15. Dolhnikoff M, da Silva LF, de Araujo BB, *et al.* The outer wall of small airways is a major site of remodeling in fatal asthma. *J Allergy Clin Immunol* 2009; 123:1090–1097.
16. Pechkovsky DV, Hackett TL, An SS, *et al.* Human lung parenchyma but not proximal bronchi produces fibroblasts with enhanced TGF-beta signaling and alpha-SMA expression. *Am J Respir Cell Mol Biol* 2010; 43:641–651.
This study demonstrated the heterogeneous molecular and biophysical characteristics of fibroblasts isolated from normal human proximal bronchi and distal lung parenchyma.
17. Leuppi JD, Salome CM, Jenkins CR, *et al.* Markers of airway inflammation and airway hyperresponsiveness in patients with well controlled asthma. *Eur Respir J* 2001; 18:444–450.
18. Sigurs N, Aljassim F, Kjellman B, *et al.* Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 2010; 65:1045–1052.
19. Goldman MD, Nazeran H, Ramos C, *et al.* Electrical circuit models of the human respiratory system reflect small airway impairment measured by impulse oscillation (IOS). *Conf Proc IEEE Eng Med Biol Soc* 2010; 2010:2467–2472.
20. Keen C, Olin AC, Wennergren G, Gustafsson P. Small airway function, exhaled NO and airway hyper-responsiveness in paediatric asthma. *Respir Med* 2011; 105:1476–1484.
This study revealed a positive association between small airway dysfunction and inflammation in pediatric asthma.
21. Kelly VJ, Brown NJ, King GG, Thompson BR. A method to determine in vivo, specific airway compliance, in humans. *Med Biol Eng Comput* 2010; 48:489–496.
This study is the first to describe a method to measure and quantify airway compliance in humans.
22. Mugler JP III, Altes TA, Ruset IC, *et al.* Simultaneous magnetic resonance imaging of ventilation distribution and gas uptake in the human lung using hyperpolarized xenon-129. *Proc Natl Acad Sci U S A* 2010; 107:21707–21712.
Patients with lung disease were assessed by MRI, which demonstrated underlying pathological alterations in lung tissue and local blood flow.
23. de Lange EE, Altes TA, Patrie JT, *et al.* Evaluation of asthma with hyperpolarized helium-3 MRI: correlation with clinical severity and spirometry. *Chest* 2006; 130:1055–1062.
24. Campana L, Kenyon J, Zhalahdoust-Sani S, *et al.* Probing airway conditions governing ventilation defects in asthma via hyperpolarized MRI image functional modeling. *J Appl Physiol* 2009; 106:1293–1300.
25. Tustison NJ, Altes TA, Song G, *et al.* Feature analysis of hyperpolarized helium-3 pulmonary MRI: a study of asthmatics versus nonasthmatics. *Magn Reson Med* 2010; 63:1448–1455.
Hyperpolarized helium-3 MRI was used to assess 27 spirometric features in 55 individuals (47 asthmatic and eight normal) before and after respiratory challenge. This technique provided an excellent appraisal of regional lung ventilation with good discriminatory power between healthy and asthmatic patients.
26. Vanden Burgt JA, Busse WW, Martin RJ, *et al.* Efficacy and safety overview of a new inhaled corticosteroid, QVAR (hydrofluoroalkane-beclomethasone extrafine inhalation aerosol), in asthma. *J Allergy Clin Immunol* 2000; 106:1209–1226.
27. Gentile DA, Skoner DP. New asthma drugs: small molecule inhaled corticosteroids. *Curr Opin Pharmacol* 2010; 10:260–265.
28. Hoshino M. Comparison of effectiveness in ciclesonide and fluticasone propionate on small airway function in mild asthma. *Allergol Int* 2010; 59:59–66.
The findings from this study on 30 mild asthmatic patients suggest that a small particle corticosteroid, ciclesonide, improves small airway function and inflammation better than fluticasone propionate.
29. Scichilone N, Battaglia S, Sorino C, *et al.* Effects of extra-fine inhaled beclomethasone/formoterol on both large and small airways in asthma. *Allergy* 2010; 65:897–902.
This pilot study on 30 asthmatic patients demonstrated that treatment with extra-fine inhaled beclomethasone/formoterol had beneficial effects on both large and small airways function.
30. Kraft M, Cairns CB, Ellison MC, *et al.* Improvements in distal lung function correlate with asthma symptoms after treatment with oral montelukast. *Chest* 2006; 130:1726–1732.
31. Fritscher LG, Rodrigues MT, Zamel N, Chapman KR. The effect of montelukast on exhaled nitric oxide of alveolar and bronchial origin in inhaled corticosteroid-treated asthma. *Respir Med* 2009; 103:296–300.
32. Ghio L, Zanconato S, Rampon O, *et al.* Effect of montelukast added to inhaled corticosteroids on fractional exhaled nitric oxide in asthmatic children. *Eur Respir J* 2002; 20:630–634.