

STATE OF THE ART REVIEW

Provoked models of asthma: what have we learnt?

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Clinical and Experimental Allergy

Summary

Asthma is a chronic inflammatory disease of the airways characterized by physiological abnormalities of variable airflow obstruction and airway hyperresponsiveness (AHR) to a wide variety of physical and inhaled chemical stimuli and the presence of symptoms. AHR is measured by challenging the airways with a variety of agonists and naturally occurring stimuli, which results in constriction of the airway smooth muscle, leading to airway narrowing and airflow limitation. There are two distinct mechanisms by which the airways can narrow to a constrictor stimulus and these are defined by the pathways they take to induce AHR. Direct stimuli are pharmacological agents administered exogenously (such as histamine or methacholine) that act 'directly' on specific receptors on the bronchial smooth muscle to cause constriction. The other mechanism by which the airway can narrow is via the inhalation of indirect stimuli, which include natural stimuli, such as allergen or exercise, and pharmacological agents such as adenosine monophosphate and hyper-osmotic agents (e.g. hypertonic saline or dry powder mannitol). These stimuli induce airway narrowing 'indirectly' by causing the endogenous release of mediators of bronchoconstriction from airway inflammatory cells. Provoked models of asthma have been extremely valuable in understanding the pathobiology of asthma, in aiding diagnosis, in helping to clarify the mechanisms of actions of effective drugs and in the development of new entities to treat asthma. Some provoked models are valuable clinically, particularly those that measure direct AHR, while others, particularly allergen challenge, have been used in animal models and in humans to study the mechanisms of allergen-induced airway inflammation and the associated physiological changes, as well in the development of new drugs for asthma. An emerging role for measurements of AHR is in the evaluation of the optimal treatment for patients with asthma.

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Cite this as: P. M. O'Byrne, G. M. Gauvreau and J. D. Brannan, *Clinical and Experimental Allergy*, 2009 (39) 181–192.

Introduction

Asthma is a chronic inflammatory disease of the airways that is characterized by physiological abnormalities of variable airflow obstruction and airway hyperresponsiveness (AHR) to a wide variety of physical and inhaled chemical stimuli and the presence of symptoms, such as dyspnoea, cough, chest tightness and wheezing. Important insights into the pathogenesis of asthma were obtained in the early 1980s, when the central role of airway inflammation was identified to be important in asthma pathogenesis, even in very mild disease [1, 2]. This changed the focus in asthma management from the relief of symptoms with frequent use of inhaled short-acting β_2 -agonists (such as salbutamol or terbutaline) to the prevention of symptoms and exacerbations by the regular use of anti-inflammatory medications, particularly inhaled corticosteroids (ICS) [3].

Many of the advances in understanding asthma pathogenesis have originated from studies of provoked models of airway inflammation in both animals and humans. This review will focus on the value (and limitations) of provoked models of bronchoconstriction and/or airway inflammation in human subjects in both improving understanding of asthma, as well as in developing new drugs for this condition.

Airway hyperresponsiveness

AHR can be defined as a sensitivity of the airways to a variety of natural or pharmacological stimuli. This is measured by challenging the airways with a variety of constrictor agonists and naturally occurring stimuli, which results in constriction of the airway smooth muscle, leading to airway narrowing and airflow limitation. AHR

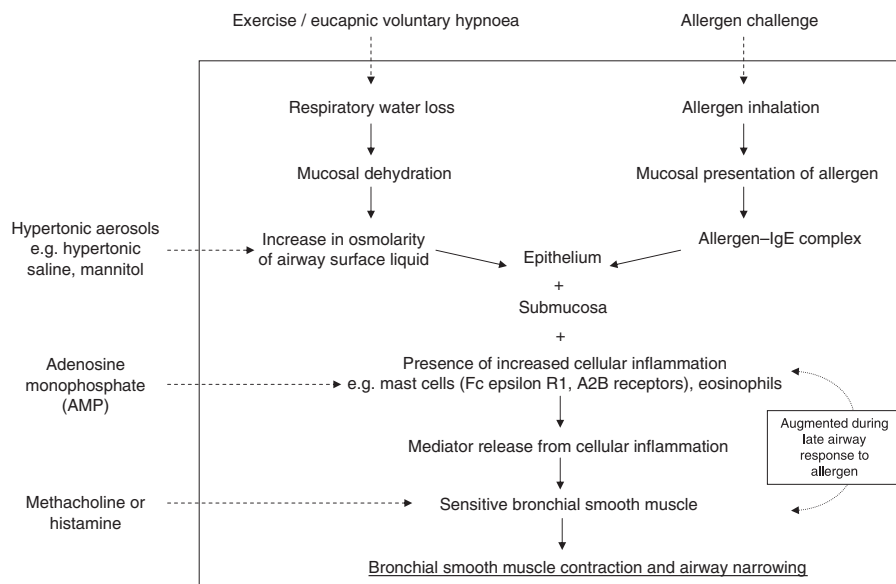


Fig. 1. A schematic demonstrating the mechanism of action of common bronchoconstricting stimuli delivered as standardized bronchial provocation tests in the research and clinical setting.

is a key feature of asthma [4]. Tests for AHR originated from research into the mechanisms of airway narrowing and later in the assessment of therapies in the treatment of asthma by protecting against AHR. While most tests for AHR have a role in research, some have use in the clinic for aiding the diagnosis of asthma [5], with an emerging role in monitoring therapy [6].

There are two distinct mechanisms by which the airways can narrow to a constrictor stimulus and these are defined by the pathways they take to induce AHR (Fig. 1). Direct stimuli are pharmacological agents administered exogenously and that act 'directly' on specific receptors on the bronchial smooth muscle to cause constriction. These include agents that have a clinical use, such as methacholine [7] and histamine [8], which act on muscarinic and histamine (H_1) receptors, respectively. Furthermore, eicosinoid mediators such as inhaled prostaglandins [9] or thromboxane [10], or cysteinyl leukotrienes [11] are useful for investigating their receptor-mediated actions on airway smooth muscle, although their role is confined to research.

When these agonists are inhaled, a partial dose-response curve is generated in asthmatics, while a more complete dose-response curve can be obtained in normal subjects (Fig. 2). The inhaled dose at which bronchoconstriction begins is called the 'airway sensitivity', the slope of the subsequent dose-response the 'airway reactivity' and, in asthmatics, the plateau response cannot be measured, while it often can be in normals (Fig. 2). In asthmatics, the airway sensitivity, reactivity and maximal responses are all enhanced, and the term 'airway responsiveness' has been used to reflect all of these. In almost all instances, the airway response is measured by changes in

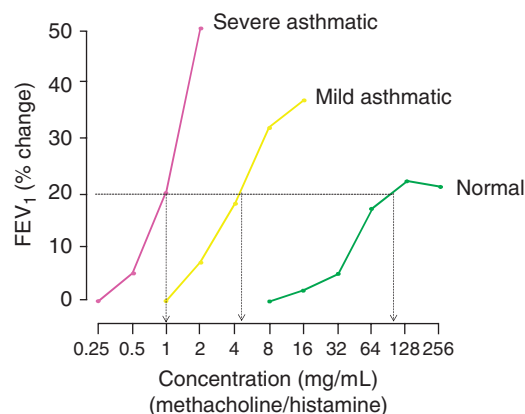


Fig. 2. Dose-response (D-R) curves to inhaled direct agonists (histamine or methacholine) in a non-asthmatic subject and patients with mild and severe asthma. The response is measured by a change in forced expiratory volume in 1 s (FEV_1). Patients are more sensitive to the agonist (as demonstrated by a shift to the left D-R curve) and more reactive (as demonstrated by a steeper slope of the curve). Also, asthmatics have a greater maximal bronchoconstrictor response. Airway hyperresponsiveness is expressed as the provocative concentration of the agonist causing a 20% decline in the FEV_1 (PC_{20}), as shown by the dashed line.

the forced expiratory volume in 1 s (FEV_1), and no further inhaled concentrations of the agonist are administered once a 20% decline in the FEV_1 has been achieved (Fig. 2). Airway responsiveness is expressed as the provocative concentration (PC) or the provocative dose (PD) of the stimuli that causes a 20% reduction in FEV_1 (Fig. 2).

The other mechanism by which the airway can narrow is via indirect stimuli, which include natural stimuli, such as allergen [12] or exercise [13] (Fig. 1). They also include pharmacological agents such as adenosine monophosphate

(AMP) [14] and hyper-osmotic agents (e.g. hypertonic saline [15] or dry powder mannitol [16]). These stimuli induce airway narrowing 'indirectly' by causing the endogenous release of mediators of bronchoconstriction from airway inflammatory cells. While the precise mechanism of each stimulus that causes mediator release may differ, they are dependent on the presence of inflammation, in particular, the mast cell and possibly the eosinophils. The response is also determined by the airway smooth muscle sensitivity to these mediators [17].

There is accumulating evidence correlating the magnitude of the responses to both direct [18] and indirect AHR [19] to the presence of inflammation in persons with clinically defined asthma. However, the correlations to direct measures of AHR are usually not strong [18], and the airway sensitivity to indirect AHR may better reflect the level of airway inflammation [20], which appears to be logical when considering the mechanism (Fig. 3).

Direct bronchial provocation tests

Protocols for measuring AHR using direct agents or pharmacological stimuli, such as methacholine and histamine, have been standardized [7] and used in clinical trials for assessing the efficacy of therapeutic agents [21–23]. AHR to direct stimuli have been shown to be associated with the severity of asthma [8] and the amount of treatment needed to treat the disease [24]. In addition, a treatment strategy aimed at reducing methacholine AHR resulted in a lower rate of mild exacerbations than did patients in a reference strategy group and a greater improvement in the FEV₁ [6]. Also, in airway biopsies there was a significant reduction in the thickness of the subepithelial reticular layer in the AHR strategy group.

AHR to inhaled methacholine can also be demonstrated in chronic obstructive pulmonary disease (COPD) patients with airflow obstruction [25]. The degree of airflow obstruction correlates with the increase in airway responsiveness, which suggests that AHR is a result of reduced airway calibre in COPD patients. Further, the results from the Lung Health Study [26] indicate that many patients who smoke, and have early COPD, have AHR that is strongly related to gender (nearly twice as many women as men: 46.6% and 23.9%, respectively), to low baseline lung function and, to a lesser extent, to respiratory symptoms. In addition, in smokers with airflow obstruction who quit smoking, the subsequent rate of decline in FEV₁ was half the rate of that in continuing smokers. The predictors of change in lung function included methacholine AHR [27]. By contrast, many patients with AHR and asthma have normal airway calibre at the time of an inhalation challenge. Indeed, measurements of direct AHR are particularly useful in patients with normal airway calibre in whom a diagnosis of asthma is being considered [5].

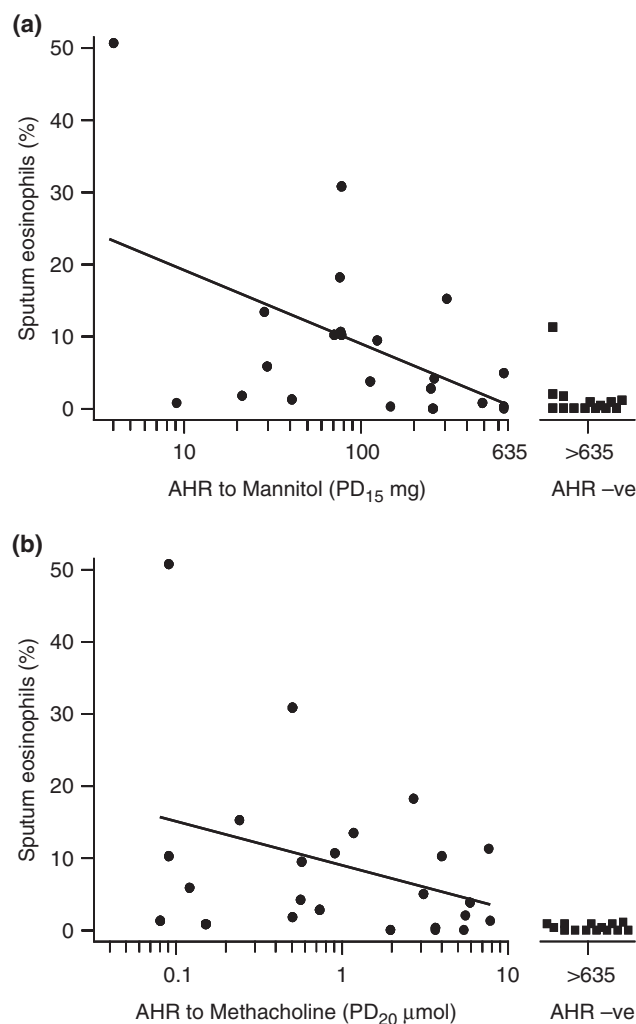


Fig. 3. The relationship between the airway sensitivity to inhaled mannitol (a) or methacholine (b) compared with the percentage (%) of eosinophils in sputum in steroid-naïve asthmatic subjects. Airway hyperresponsiveness (AHR) to mannitol is defined as a 15% reduction in the forced expiratory volume in 1 s (FEV₁) to a cumulative dose of < 635 mg (PD₁₅), and for methacholine as a 20% reduction in FEV₁ to < 8 µmoles (PD₂₀). The relationship of % eosinophils with those who had a positive AHR (closed circles) was significant for mannitol ($r_p = -0.52$, $P < 0.05$) compared with methacholine ($r_p = -0.28$, $P = \text{NS}$). Those who had no AHR to either mannitol or methacholine (closed squares) in this group of patients had significantly less sputum eosinophils (Adapted from reference [19]).

Measurements of direct AHR have been helpful in studies of asthma genetics. There is familial clustering for asthma and for AHR [28], and studies of monozygotic and dizygotic twins have suggested that some genetic basis exists for the development of AHR [29], but that environmental factors are likely more important. Reports of genetic linkage have identified associations between direct AHR and several genetic markers on chromosome 5q, near a locus that regulates serum IgE levels [30]. Another study has identified a linkage between a highly polymorphic marker of the FcεR1β subunit of the high-affinity

IgE receptor on chromosome 11q and methacholine AHR, even in patients with non-atopic asthma [31]. Also, one gene polymorphism (Glu 27), of the nine identified of the β_2 -adrenoceptor, has also been associated with increased methacholine AHR [32], whereas another polymorphism (Gly 16) was associated with the presence of nocturnal asthma [33]. A number of other genetic abnormalities have been linked to the presence of AHR. These include polymorphisms in a recently identified asthma susceptibility gene, GPRA (G-protein-related receptor for asthma), which increases the susceptibility for asthma and AHR [34], and a polymorphism in the gene for a disintegrin and metalloprotease 33 (ADAM33) [35].

AHR has been correlated to a number of markers of persisting airway inflammation in asthma. These include the number of eosinophils [1], mast cells [1] and epithelial cells in bronchoalveolar lavage fluid [2], and the extent of deposition of collagen and other ECM proteins below the basement membrane in asthmatic airways [36]. In addition, a number of other structural changes have been described in asthmatic airways that appear to be characteristic of the disease and may also be responsible for the presence of persisting AHR in asthma. These include patchy desquamation of the epithelium [36], airway smooth muscle hypertrophy and hyperplasia [37], and vascular hyperplasia [38]. These pathological changes have been implicated in the pathogenesis of AHR in asthma and are even present early and in mild forms of the disease [39]. These observations suggest that persisting AHR in asthma is caused by inflammatory and structural changes in the airways (remodelling). However, not all studies have associated the presence of airway inflammation with AHR [40]. It is most plausible that the airway structural change that is most important in causing AHR is the increase in airway smooth muscle [41], as airway smooth muscle is the structure that controls airway calibre. However, it is not certain whether there is a change in the intrinsic properties of airway smooth muscle, a change in the structure and/or the mechanical properties of the non-contractile components of the airway wall or a change in the interdependence of the airway wall with the surrounding lung parenchyma [42]. Whatever the precise mechanism responsible for AHR in asthma, it cannot be used as a direct surrogate of airway inflammation or airway remodelling, as it is likely a composite of both.

Increases in direct AHR associated with increased asthma symptoms have been associated with exposure to pro-inflammatory stimuli, particularly environmental allergens [43, 44] or occupational sensitizers [45]. These changes are usually transient and recover once the exposure to the stimulus is over [46]; however, as has been shown with occupational exposure to Western Red Cedar dust, prolonged occupational sensitizer exposure can result in irreversible AHR [47]. This again has been taken

as indirect evidence that airway inflammation can cause transient direct AHR, but more prolonged inflammation may result in airway remodelling and persisting direct AHR.

Measurement of direct AHR has also been used to assess current therapies for the treatment of asthma. The inhibition on AHR by these therapies has in turn provided some explanation as to the mechanisms of direct AHR. Regular use of ICS is well known to attenuate direct AHR [48, 49]; however, this does not often improve to the normal (non-asthmatic) range, and can take many months to years to normalize [50]. The improvements with ICS on direct AHR suggest that short-term changes are related to reductions in airway inflammation. More long-term improvements may be suggestive of improvements in airway remodelling; however, this is not well defined.

Acutely, β_2 -agonists are powerful inhibitors at decreasing the smooth muscle sensitivity of agents that induce direct AHR [51]. The mechanism of action is functional antagonism of the bronconstrictor stimulus via β_2 receptor-induced airway smooth muscle relaxation. The methacholine challenge is a recognized methodology for assessing the efficacy and duration of action of bronchoprotection and the pharmacoequivalence of a β_2 -agonist [22]. However, patients who regularly use β_2 -agonists may show increased airway sensitivity to both direct and indirect AHR, translating to a decreased bronchoprotection [52–54].

The acute use of a leukotriene antagonist [55] and mast cell stabilizers such as sodium cromoglycate [56] and nedocromil sodium [57] demonstrates minimal effect to no effect on AHR to direct stimuli. This suggests that there are limitations in using direct AHR to investigate the bronchoprotective effect of therapies that involve the specific antagonism via individual receptors on the bronchial smooth muscle or for agents that stabilize the mast cell.

Indirect bronchial provocation tests

Indirect stimuli include allergen or stimuli such as exercise, dry air hyperpnoea, AMP or osmotic aerosols. Antigenic stimuli are confined to research, while the others have applications clinically, and these differences provide unique opportunities when investigating a specific bronchoprotective or anti-inflammatory action of a pharmacotherapy.

Exercise-induced bronchoconstriction (EIB) is a common feature of asthma and a real-life stimulus that can occur in patients with current asthma [58]. The magnitude of EIB correlates with the degree of direct AHR in persons with clinically active asthma [59]. Therefore, in some patients with mild episodic asthma, and only mildly increased airway responsiveness, even strenuous exercise does not cause bronchoconstriction. In some instances,

this may be due to the use of regular ICS [60]. Occasionally, EIB can be the only manifestation of asthma. Exercise or dry air hyperpnoea causes airway narrowing because of the evaporative and cooling effects of respiratory water loss [13]. This results in an increase in the osmolarity of the airway surface, which causes the release of bronchoconstricting mediators such as histamine [61], prostaglandins [62] and leukotrienes [63]. The inhalation of hypertonic aerosols has been demonstrated to be effective in identifying persons susceptible to having EIB [64] as well as in demonstrating the efficacy of therapies both in the acute and in the long-term inhibition of EIB and in the treatment of asthma [65, 66].

In many asthmatic patients with EIB, the episode of bronchoconstriction is followed by a period during which exercise causes less bronchoconstriction, and that with repeated bouts of exercise the bronchoconstriction can be abolished [67]. Several mechanisms have been proposed to explain exercise refractoriness, with the most probable being the cysteinyl leukotriene-stimulated release of inhibitory prostaglandins that partially protect the airways [68]. PGE_2 acts as a functional antagonist by increasing intracellular cyclic AMP (cAMP) in airway smooth muscle, as an inhibitor of mediator release or through an as-yet-unexplained mechanism. Thus, the studies of the mechanisms of EIB helped identify an important role for the cysteinyl leukotrienes and inhibitory prostanoids in asthma.

Protocols for exercise are standardized and have been used in the evaluation of therapies for their acute protective effect on EIB in persons with asthma [69]. Subjects exercise vigorously breathing a source of dry air and a sufficient level of ventilation should be reached and sustained by either running or cycling for 6–8 min to induce adequate airway dehydration. An abnormal response is a 10–15% decline in FEV_1 after exercise, measured periodically at least 15–20 min after exercise (Fig. 4). Following an exercise challenge, the reproducibility of the % decline in FEV_1 is greater than that of the area under the post-exercise FEV_1 vs. time curve and the power calculations for sample sizes for these outcomes are available [70].

Surrogate tests for EIB have been developed that have practical and safety advantages for clinical use. These surrogate tests for EIB include eucapnic voluntary hyperventilation (EVH) [71] and osmotic stimuli delivered either as an aerosol of hypertonic saline or mannitol [64]. Both these tests have been derived from the understanding of the mechanism of EIB.

EVH testing, first developed by the US Army to screen recruits, is clinically useful in persons who have normal lung function and who are suspected of having EIB alone, such as athletes [72]. The advantage of this test over exercise is that it can be performed without the need to exercise the patient. Also, higher levels of ventilation can be achieved more rapidly, which is important in an athlete

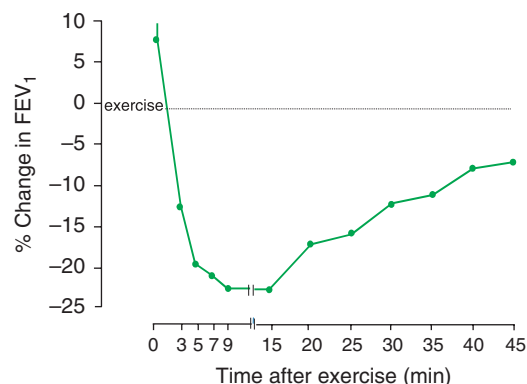


Fig. 4. The bronchoconstriction caused by exercise in an asthmatic patient. The response is measured by a change in forced expiratory volume in 1 s (FEV_1). Exercise often causes an initial bronchodilator response, but this is followed by a decline in FEV_1 that is usually maximal between 10 and 30 min after exercise.

population, who may not demonstrate AHR from a laboratory exercise challenge. However, EVH provides a 'bolus dose' of hyperventilation and can cause significant bronchoconstriction in known asthmatics and should be used with caution in this subject group.

Osmotic bronchial provocation using nebulized hypertonic saline (using a 4.5% concentration) or dry powder mannitol (AridolTM or OsmohaleTM, Pharmaxis Ltd, Frenchs Forest, NSW, Australia) have been developed to aid in further improving the ease of administration of indirect testing [73]. Mannitol provides a common operating standard that may be attractive for multi-centre studies in addition to demonstrating suitable reproducibility, comparable with direct stimuli [16, 74]. Similar to AMP and direct stimuli, osmotic challenges are dose-response challenges that do not induce significant reductions in FEV_1 and they have shown adequate safety in large populations [73].

Pharmacotherapy for the treatment of asthma has revealed much about the mechanism of indirect AHR. Acute use of β_2 -agonists demonstrates the most potent protective effect on indirect stimuli, likely due to a combination of functional antagonism of mediators causing bronchoconstriction, as well as via β_2 -receptor-mediated mast cell stabilization [75]. Drugs such as sodium cromoglycate and nedocromil sodium are also potent inhibitors of indirect AHR due to their mast cell-stabilizing properties [66, 75, 76]. Leukotriene antagonists are not as protective at inhibiting the initial bronchoconstrictive response to indirect stimuli; however, they have a powerful effect in causing a rapid recovery from indirect induced AHR [77]. Thus, the airway sensitivity and recovery from an indirect bronchoconstricting stimulus may be important to consider when assessing new pharmacotherapy.

AMP is a receptor-mediated indirect stimulus that acts by causing airway narrowing via activation of A2b

receptors on the mast cell surface, leading to mast cell degranulation and the release of bronchoconstricting mediators [78]. Unlike some other indirect challenges, such as exercise [79], AMP inhalation causes an increase in airway eosinophils [80], similar to inhaled allergen [81].

Allergen inhalation challenge via the aerosol delivery of specific allergens has been well characterized and useful for understanding the mechanisms of allergic airway inflammation [81] and AHR [46]. The rationale for using inhaled allergens to study the pathobiology of asthma is that environmental allergens have been identified in many epidemiologic studies as an important cause of asthma [82]. Environmental allergens are considered inducers of asthma (as are respiratory viruses and occupational sensitizers) rather than just inciters of asthma symptoms. More than 80% of children with asthma are sensitized to environmental allergens, and some allergens, particularly those found within the household environment, are particularly implicated [82, 83]. Indeed, the fact that inhaled allergens cause bronchoconstriction, airway inflammation and direct AHR has been used as the rationale for developing many of the animal models, which have been used to study the immunology of allergic asthma or the development of new drugs to treat asthma.

The administered allergen cross-links antigen-specific IgE that is bound to IgE receptors on mast cells resident in the airway and circulating basophils. This is followed by mast cell degranulation and up-regulation of eicosinoid pathways, which releases a variety of preformed (e.g. histamine) [84] and newly formed mediators (e.g. leukotrienes, prostaglandins) [85, 86] of bronchoconstriction, which also lead to increasing vascular permeability. The immediate onset of bronchoconstriction by these mediators constitutes the early airway response (EAR) and can reach a maximum at 30 min after inhalation of allergen and resolve within 3 h. However, in approximately half the individuals who have an EAR, a late airway response (LAR) can result, generally between 4 and 8 h [12], with some reports of this occurring beyond this period (Fig. 5).

Individuals with dual allergen-induced responses (both early and late responses) have been particularly interesting to study. This is because the late response is mechanistically different and results from an increase in the recruitment of cellular inflammation (both basophils and eosinophils) [81], vascular permeability and mucous secretion. Also, the late response has been shown to be associated with an increase in AHR to stimuli, such as methacholine [12], exercise [87] and AMP [88]. This model of allergic bronchoconstriction has been useful in humans for exploring the time-course of cellular inflammation and the associated physiological changes, particularly related to eosinophils [81], basophils [89] (Fig. 6) and dendritic cells [90, 91]. It has also been useful to document changes in cells in the airways that are thought to be important in airway remodelling, such as myofibroblasts

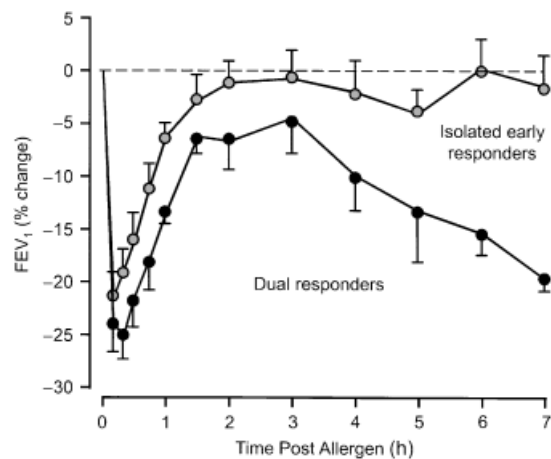


Fig. 5. Percent change in forced expiratory volume in 1 s (FEV_1) for 7 h after allergen inhalation challenge in subjects with an isolated early asthmatic response (open circles) and in subjects with a dual asthmatic response (solid circles). (Reprinted with permission from reference [93].)

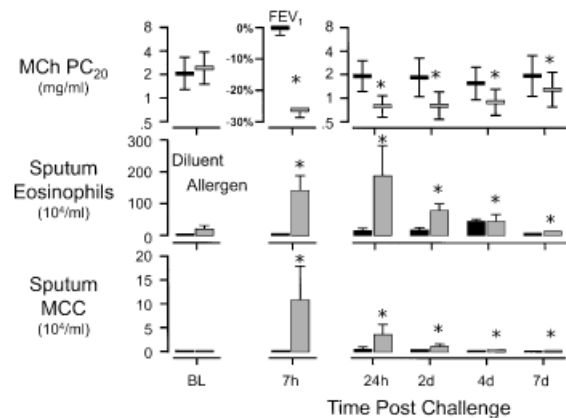


Fig. 6. Methacholine PC_{20} and maximum percent late fall in forced expiratory volume in 1 s (FEV_1) (top panel), sputum eosinophils (middle panel), and metachromatic cells (bottom panel) in response to diluent (open bars) and allergen (stippled bars) inhalation challenge. All data are expressed as mean and SEM, except for methacholine PC_{20} which is expressed as geometric mean and geometric SEM. (Reprinted with permission from reference [81].)

[92]. In addition, this model has allowed the evaluation of the mechanisms of bone marrow production of eosinophils and basophils [93, 94], and release [95] in response to an allergen delivered into the airways. The studies have shown that up-regulation of IL-3 receptor-positive progenitors occurs very soon after allergen inhalation (within 7 h), while IL-5-positive progenitors are seen within 24 h [94], and that increased production of IL-5, needed for eosinophil differentiation in the bone marrow, is provided by increased numbers of IL-5-producing T cells, possibly originating from the airways [96].

Allergen inhalation challenge has also been useful in evaluating the efficacy of anti-inflammatory therapies in allergic asthma, both of currently used therapies, as well as new therapies. It is, by far, the most widely used animal

model for screening molecules for airway anti-inflammatory activity in pharmaceutical development. In addition, all the currently approved drugs used to treat asthma modify, in some way, allergen-induced airway responses. These include both short-acting [97] and long-acting inhaled β_2 -agonists [98] (by acting as functional antagonists); ICS [99, 100] (likely through a variety of anti-inflammatory mechanisms); anti-leukotrienes [101–103] (through inhibiting the release or action of cysteinyl leukotrienes); cromones [104, 105] (through inhibiting mast cell degranulation); anti-IgE [106, 107]; methylxanthines [108] (likely as weak functional antagonists); and prednisone [109]. However, not all drugs that attenuate allergen-induced responses have proven to be effective anti-asthma medications [110, 111]. By contrast, there is no example of a drug that has not attenuated allergen-induced airway responses and that has subsequently proven to be effective in asthma. There are many examples of drugs evaluated in Phase IIa studies using an allergen inhalation challenge that have failed in this model, but that have subsequently also been evaluated in much larger Phase IIb studies in patients with symptomatic asthma and that have also failed. These include the esterase-sensitive ICS [112], PAF antagonists [113], thromboxane antagonists [114], oral anti-allergics, such as ketotifen [115], immunostimulatory sequences [116] and others, which, unfortunately, because of the bias against publishing negative studies, are not available in the archival literature. More recently, drugs that have demonstrated attenuation of allergen-induced responses in asthmatics include pitrakinra, an IL-4 variant that inhibits the binding of IL-4 and IL-13 to the IL-4 receptor [117], and a monoclonal antibody directed against IL-13 [118]. However, neither of these drugs has still been evaluated in larger clinical trials in asthmatics. Taken together, these studies suggest that an allergen-inhalation challenge in mild asthmatic subjects is a method for evaluating possible new drugs in asthma, which has a very high negative predictive value and a reasonable positive predictive value [119] (Table 1).

Viral respiratory infections

Acute viral infections have been demonstrated to be temporally associated with a number of important clinical consequences including initiating acute exacerbations of asthma both in children and in adults [120] and inducing short- and long-term alterations in airway physiology, including increasing AHR [121]. The common cold virus, the rhinovirus (RV), has been the most frequently implicated in these outcomes. Although RV has been considered to be primarily an upper respiratory tract pathogen, studies have demonstrated that it can invade the lower respiratory tract and undergo viral replication in this environment [122].

Experimental RV inoculation studies have been used to study the effects of viral infections in asthma. For example, RV can enhance both the immediate and the late-phase responses, histamine release [123] and eosinophil recruitment after inhaled allergens [121, 124]. Experimental RV infections have not been used to evaluate the mechanisms of drug effects in asthma.

Safety of provoked models

Provoked models of asthma are extremely safe, when conducted by laboratories that are experienced in their use and understand the limitations of the procedures, particularly in minimizing the risks of inducing severe bronchoconstriction. There have been no reported serious or fatal reactions to direct challenges. Indirect challenges may be more difficult to control with regard to the magnitude of bronchoconstriction induced, and this is particularly true of inhaled allergens or occupational sensitizers, although this is less of a problem for mannitol or AMP challenges, where increasing doses of the reagent are administered. There has been one case-report of an asthma fatality in a patient sensitized to the occupational sensitizer, toluene diisocyanate, who was exposed in the workplace [125]. In our experience of 965 individual research allergen inhalation challenges conducted in

Table 1. Examples of currently available and newly developed drugs for asthma on allergen-induced airway responses in mild asthmatic subjects

True positives	True negatives	False positives	False negatives
All conventional inhaled corticosteroids (ICS)	Esterase-sensitive 'soft' steroids	Anti-CD11a hMab	None to date
Short-acting inhaled β_2 -agonists	Platelet-activating factor antagonists	Inhaled PGE ₂	
Long-acting inhaled β_2 -agonists (LABA)	Inhaled leukotriene receptor antagonists	PGE ₁ analogue	
Combination ICS/LABA	Thromboxane antagonists		
Oral leukotriene receptor antagonists	Ketotifen		
Anti-IgE			
Theophylline	Immunostimulatory sequence		
Prednisone			

True positives are drugs that attenuate allergen-induced responses and are effective in asthma; true negatives are drugs that did not attenuate allergen responses and failed clinical trials in asthma; false positives are drugs that attenuated allergen responses, but may not be useful in asthma; and false negatives are drugs that did not attenuate allergen responses and were effective in asthma.

collaboration with a number of other centres in Canada between 2003 and 2008, there has not been any hospitalization or fatality, and only four subjects required the initiation of ICS for several days, and one subject required oral corticosteroids because of a more severe late response. However, it must be stressed that these tests were conducted by individuals highly experienced in these challenge procedures.

Limitations of provoked models

While provoked models have been very helpful, for the reasons enumerated above, in understanding asthma pathogenesis, the mechanisms of action of effective anti-asthma medications, as well as in new drug development, all the models measure a component of the asthma phenotype and none capture the complexity of what is a very heterogeneous disease, in terms of both its clinical presentation and pathogenesis. There is, for example, no model that allows the study of non-atopic, late-onset asthma (although some of these patients have occupational asthma), severe steroid-resistant asthma, the progression of asthma or the early origins of the disease. Also, some of the challenge models result in a transient increase in symptoms and medication use. The transient nature of the changes is different from the persistence of symptoms experienced by many asthmatics, and yet the fact that there is a change in asthma control means that the challenges need to be performed by laboratories that are experienced in the methodology used. This is particularly true for allergen and occupational challenges, and experimental RV infection. Finally, lack of regulatory approval, for many of the direct challenges (with the notable exception of inhaled methacholine) and indirect challenges (except inhaled mannitol in some countries), without a GMP label and completion of safety studies, has limited the use of these challenge tests in almost all countries, except in the research setting.

Conclusions

Provoked models of asthma have been extremely valuable in understanding the pathobiology of asthma, in aiding diagnosis, in helping clarify the mechanisms of actions of effective drugs and in the development of new entities to treat asthma. All the models have reported standardized methods, and the reliability of the methods is (in general) known. These models have different uses both clinically and in research. Some are valuable clinically, particularly those that measure direct AHR, while some tests for indirect AHR have practical advantages demonstrating clinical utility. Allergen challenge has been extensively used in animal models and humans to study the mechanisms of allergen-induced airway inflammation and the

associated physiological changes, as well as in the development of new drugs for asthma.

Looking to the future use of provoked models in asthma, it is likely that the direct challenges will remain a mainstay for asthma diagnosis and may have a more prominent role in helping in decision making about treatment, such as when to change doses of anti-inflammatory treatment, or even stop treatment if airway responsiveness has been normalized. It is also likely that some of the indirect challenges (such as mannitol) will be evaluated in the same way in research studies, and it would be of great interest to know whether the value of using direct or indirect challenges provides the same information about these clinical management decisions. The provoked models that use allergen inhalation are gaining credibility as a useful way to evaluate the possible benefit of new anti-inflammatory treatment approaches, but are unlikely to ever be a useful tool for clinical decision making.

Acknowledgements

Potential for conflict of interest: P. O' B. has been on Advisory Boards for AstraZeneca, Biolipox, GSK, Merck, Nycomed, Topigen, Resistentia and Wyeth, and has received lecture fees from these and other pharmaceutical companies including Chiesi and Ono Pharma. In addition, he has received grants-in-aid for research studies from AstraZeneca, Boehringer Genentech, GSK, Medimmune, Merck, Pfizer and Wyeth. G. M. G. has received grants-in-aid for research studies from AstraZeneca, Boehringer Genentech, GSK, Medimmune, Merck, Pfizer and Wyeth. J. D. B. was employed by the South West Sydney Area Health Service (SWSAHS), which is the owner of the intellectual property describing the use of mannitol for bronchial provocation, which has been licensed to Pharmaxis Ltd, and is a licensed test for AHR in Australia and the European Union known as AridolTM or OsmohaleTM. The SWSAHS will receive royalties from Pharmaxis Ltd for the sale of Aridol/Osmohale following the reimbursement of development costs. The Department of Respiratory Medicine at Royal Prince Alfred Hospital, S. D. A. and J. D. B. may be in the position in the future to benefit from a distribution of a royalty. J. D. B. purchased the minimum allotment of 12 500 shares in Pharmaxis Ltd J. D. B. will consult for Pharmaxis Ltd when requested and will receive fees for this consultation.

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