

Review article

The allergen bronchoprovocation model: an important tool for the investigation of new asthma anti-inflammatory therapies

Allergen bronchoprovocation tests have been used for more than two decades in the investigation of respiratory allergic diseases such as asthma and rhinitis. These bronchial challenges are now well standardized and can offer key information on the therapeutic potential of new agents and on their anti-inflammatory effects on the airways. Both standard and low-dose allergen provocations are safe when performed by experienced investigators and do not lead to persistent worsening of asthma or change in airway function. The evaluation of new therapeutic agents by these methods can also provide important information on the mechanisms of development and persistence of airway diseases.

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The prevalence of asthma and allergic diseases has been increasing worldwide in the last few decades (1). Asthma is usually defined as a disease of the conducting airways characterized by variable airway obstruction and hyperresponsiveness (AHR; 2). Its development as its clinical expression, is considered to be due to an underlying airway inflammatory process and to bronchial structural changes (3). Classically, airway inflammation, which can be found in asthma of all severities, is a T_H2-mediated process, with cytokines and mediators release, involved in bronchoconstriction, plasma transudation, vasodilatation, mucus hypersecretion, and structural changes (4–6). In mild-to-moderate asthma, this process is

responsive to anti-inflammatory agents such as inhaled corticosteroids (ICS); these drugs are being therefore considered as first-line controller therapy for this disease.

Exposure to allergens is an important factor that may lead to the development of asthma in sensitized subjects (7). Following the initial contact with an allergen, the process of sensitization may occur in genetically predisposed individuals, with the synthesis of immunoglobulin (Ig) E antibodies. Clinical expression of atopy will vary and may involve organs such as the airways, the nose, the gut, and the skin. Allergic rhinitis often precedes asthma (8). Chronic exposure to allergens, particularly indoors such as animal danders and house dust mites, may lead, through an airway inflammatory and remodeling process, to the development of symptomatic asthma (9, 10). Once asthma is established, subsequent allergen exposure may trigger an asthmatic response associated with an increase in airway inflammation.

In a sensitized subject, antigen interaction with mast cells-bound IgE antibodies will result in the release of

Abbreviations: AHR, airway hyperresponsiveness; DAR, dual asthmatic response; EAR, early asthmatic response; FEV₁, forced expiratory volume in 1 s; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, increased expression of intercellular adhesion molecule-1; ICS, inhaled corticosteroids; LAR, late asthmatic response; LDAC, low-dose allergen challenge; PC₂₀, provocative concentration of methacholine inducing a 20% fall in FEV₁; T_{reg}, T-lymphocytes regulators.

mediators such as histamine and leukotrienes, along with numerous other substances including cytokines such as interleukin (IL)-4, IL-5, and granulocyte-macrophage colony-stimulating factor. Localized bronchial inflammation will then occur through the upregulation of various chemokines and adhesion molecules (11). Such process may result in a reduction of expiratory flows, either immediately following exposure (early response) and/or in the following hours (late response). This may be associated with an increase in nonallergic airway responsiveness (e.g. methacholine) in the following hours and days (12).

The magnitude of the asthmatic response to an allergen will mostly depend on the baseline nonallergic airway responsiveness, the level of specific IgE antibodies as determined by skin prick test or radioallergosorbent test, and the dose of allergen inhaled (13).

Interactions between the upper and lower airways have been emphasized in the last decade (14). Most patients with allergy also have an associated rhinitis and a significant number of patients with rhinitis will eventually develop asthma, suggesting that rhinitis may be a prestage of asthma in some individuals (15). Indeed, up to 30% of patients with chronic allergic rhinitis have asthma and rhinitic patients often show AHR (16). Lower airway inflammation has been demonstrated in rhinitic subjects without any evidence of asthma or AHR (17–19). In asthmatic subjects, the nasal inflammatory process shares many similarities with that of lower airways (20). Thus, the study of airway responses to allergen, either from natural exposure or in the context of well-standardized laboratory procedures, has helped and will continue to help understand the pathophysiology of asthma and can contribute to the assessment of clinical effects and mechanisms of action of new asthma medications.

Development of the allergen bronchoprovocation model

The standard allergen-inhalation challenge as it is usually performed consists of several components (21). The early asthmatic response (EAR) is an episode of bronchoconstriction, which is maximal 15–30 min after exposure and resolves by about 2 h. The late asthmatic response (LAR) is an episode of recurrent bronchoconstriction occurring between 3 and 8 h (or more) after exposure and occurs in about 50% of positive allergen challenges. Allergen-induced increase in AHR occurs between 3 h and several days following allergen exposure and is closely associated with the LAR. Allergen-induced eosinophilic and metachromatic cell airway inflammation, also lasting for up to several days, is also seen in conjunction with the LAR (22). This manuscript reviews the history, the recognition, and measurement of these four allergen-induced responses.

Early asthmatic response

Since the EAR was (indeed still is) the easiest allergen response to recognize clinically, the history of allergen provocation began with studies involving early responses. The first allergen challenge series was reported in 1873 when Charles Blackley used this approach to confirm that pollen (and other allergens) was the cause of allergic rhinitis and immediate asthmatic responses (23). A period of over 50 years elapsed before there was much in the way of published data regarding allergen challenges (24). Challenges were generally made using nebulized aqueous solutions. The response end point was either exclusively or primarily the EAR. Measurements were initially rather crude and included such things as the development of symptoms, signs (*coarse breathing*; 25, 26), or changes in maximal breathing capacity and/or vital capacity (27–31). Technology to measure expiratory flow rates including the forced expiratory volume in 1 s (FEV₁) was developed in France in the mid-1940s (31). The FEV₁ was named and its technology standardized in 1957 (32). Initial use of expiratory flow rates to measure the EAR included the somewhat subjective end points of *a clearcut reduction in expiratory flows* (33) or the French equivalent the *dose liminaire* required to produce a *tangible* spirometric change (34). The FEV₁, now the standard end point for measurement of most bronchoprovocation studies with allergen or others, became established in the late 1950s (35) and thereafter as the end point, generally expressed as a percentage change, used to monitor allergen provocation.

Late asthmatic response

The allergen-induced LAR was first described symptomatically by Blackley following his own personal accidental high exposure to grass pollen. In 1951 and 1952, Herxheimer noted that several of his subjects complained of symptoms several hours after inhalation (36, 37). Only a few of these were captured by changes in vital capacity; most were identified only based on symptoms. Herxheimer surmised that many of these represented a prolonged response with the improvement between the early and late periods being due to the administration of bronchodilator (37). Understanding of the classic biphasic nature of the allergen response, namely the EAR with spontaneous resolution followed by a LAR, became obvious over the next decade (38). By the late 1960s and early 1970s, despite important differences in opinions regarding immunopathogenesis, Professor Orie and his colleagues in the Netherlands (39–42) and Professor Pepys and colleagues in the United Kingdom (43–46) used this dual asthmatic response model to investigate the pathogenesis of asthma and study the effects of asthma therapy.

Allergen-induced airway hyperresponsiveness

Airway hyperresponsiveness, e.g. to histamine or methacholine, is a consistent essentially defining feature of asthma (47). Grass pollen seasonal increases in AHR to histamine were first observed by Roger Altounyan (48). Increased airway responsiveness to histamine and methacholine following allergen challenge in the laboratory was demonstrated by Dr Hargreave in 1977 (49). Subsequent investigations showed a very close relationship between allergen-induced AHR and the allergen-induced LAR; both occur in approximately 50% of positive allergen challenges (49, 50). This was a small but important step in understanding the pathogenesis of allergen-induced asthma. Measurement of airway responsiveness before and after allergen challenge has now become a routine component of most challenge methodologies (21).

Allergen-induced airway inflammation

Allergen-induced airway inflammation was first documented by de Monchy in 1985 using bronchoscopic lavage (51). Subjects with allergen-induced LAR demonstrated a substantial increase in eosinophils, whereas those with no response and with only an EAR did not. This can be measured less invasively by examining inflammatory cells in induced sputum (22). Airway inflammation can also be assessed indirectly by techniques such as measurement of exhaled nitric oxide (NO; 52). As for airway responsiveness, direct or indirect assessment of allergen-induced airway inflammation has now become a routine component of many allergen challenge models (53).

Methodological aspects

Although allergen-inhalation challenges are invaluable for studying the mechanisms of airway diseases, they may induce severe acute bronchoconstriction, exacerbation of asthma with recurrent nocturnal symptoms lasting for several days, or potentially anaphylaxis. These inhalational challenges must therefore be carried out carefully, with measures of safety applied throughout the procedure. The allergen dose provided should induce a sufficient airway response, but in a range that is safe for the patient.

The degree of AHR to nonallergic stimuli and the circulating levels of specific IgE are the main determinants of early-phase bronchial responsiveness to allergen (12, 54). Therefore, the starting allergen concentration for inhalation can be determined from the results of a methacholine challenge test and of the allergy skin prick tests titration with increasing concentrations of allergen extracts (55). The allergen extract selected for allergen challenge is ideally the one having the largest wheal

response on skin prick testing, or at least one with a sufficient wheal diameter to allow induction of an asthmatic response with the usual concentrations of allergen available. The lowest concentration of this causing a 2-mm skin wheal and the provocative concentration of methacholine inducing a 20% fall in FEV₁ (PC₂₀), predict the allergen PC₂₀. The starting concentration of inhaled allergen is chosen at two or three doubling doses below that predicted to induce a 20% fall in PC₂₀ as a safety measure (13, 55).

Methods of allergen inhalation have not been uniform across research laboratories. One commonly used method uses tidal breathing of doubling concentrations of allergen diluted in physiologic saline (56). If multiple challenges are carried out within the study design, sufficient aliquots of allergen can be prepared and frozen until used, thereby reducing the variability of repeated allergen preparation. With our current methodology, allergen challenges are initiated with administration of an allergen dose for a period of 2 min by tidal breathing through a Wright Nebulizer (Roxon Medi-Tech Ltd, Montreal, Canada) and Hans Rudolph valve (Hans Rudolph, Kansas City, MO, USA), with a filter placed on the expiratory side of the valve, to reduce contamination of the provocation chamber by the allergen. The starting concentration of allergen is 2–3 doubling doses below that predicted to induce a 20% fall in FEV₁ for high-dose challenges, and even lower concentrations of allergen for low-dose challenges. Subjects inhale through the mouth and wear nose clips to prevent delivery of allergen to the nose, which is important in subjects having allergic rhinitis. The nebulizer is driven by compressed air at 50 psi and the flow rate is adjusted to achieve an output of 0.13 ml/min. Nebulizers require regular calibration to ensure the desired output is maintained, and the same nebulizer is used to deliver all allergen challenges for a given subject. The aerodynamic mass median diameter of 0.5–2 µm delivers allergen to the small airways, and standardization of using the same nebulizer for all challenges ensures constant delivery between repeated challenges in crossover study designs.

The FEV₁ is measured 10 min after inhalation of the first concentration of allergen, and if there is no fall in FEV₁ the next concentration of allergen is administered. For high-dose challenges, if the FEV₁ has fallen between 15% and 20% from preallergen baseline, the FEV₁ is repeated 20 min after inhalation, and if it has fallen at least 20% from baseline the challenge is stopped. If the FEV₁ has not fallen at least 20% from baseline, the next concentration of allergen is administered. Low-dose allergen challenges (LDAC) aim for a fall in FEV₁ of 5%. This stepwise approach for both high-dose and LDACs utilizes regular monitoring of the subject, through measurements of FEV₁, and avoids the potential of inducing severe acute bronchoconstriction.

Other laboratories deliver cumulative allergen inhalations from a dosimeter using similar assessments between

allergen inhalations (12). Although single allergen dose challenges have been carried out and can also achieve the desired responses (57), safety concerns support an incremental approach where the response to allergen can be monitored between increasing doses. Any of these methodologies used to deliver allergen by inhalation induces similar early and late-phase responses.

Allergen-inhalation challenges are most commonly used as an investigative tool to understand better the pathophysiology of asthma and possible blocking effects of immunotherapy. When a new pharmaceutical agent is studied, its ability to block the early and late-phase asthmatic responses and subsequent AHR and/or airway inflammation provides evidence for drug activity in the airways. Within this study design, patients are challenged with exactly the same dose of allergen after receiving treatment with the test drug, and again after receiving treatment with placebo. The end point measurements in such studies are the maximal early and late percentage decreases in the FEV₁ and the areas under the curve for the EAR (0–2 h postchallenge) and the LAR (3–7 h postchallenge). Reproducibility of measurements shows that no more than eight subjects are usually required to show a 50% attenuation of either the EAR or LAR, with a 90% statistical power (58).

Reproducibility of allergen-induced sputum eosinophils has also been investigated. With a randomized crossover study design, the sample size predicted to be necessary to observe 50% attenuation of allergen-induced percent of eosinophils with a power of 0.95 was <10 subjects (59, 60). The allergen-inhalation challenge is also able to differentiate a single dose of an active ICS from placebo and a highly potent ICS from a weak ICS (60), making this a highly effective model for testing effect of asthma therapies on late airway responses.

Local (segmental) airway challenges have also been safely performed in subjects with asthma (61–63). This method requires direct instillation of allergen to selected segmental airways using a bronchoscope. Patients are pretreated with nebulized salbutamol, atropine and midazolam, and oxygen is delivered via nasal cannulae throughout the procedure. Oxygen saturation and heart rate are also monitored for safety purposes. The allergen extract used for segmental allergen challenge is the one having the largest wheal response on skin prick testing, and the concentration used for the endobronchial challenge is one-tenth of that which elicited a skin wheal with a diameter of 3 mm during a skin wheal dose–response series. This method is particularly useful for assessing inflammation in restricted areas of the airway, but cannot assess the EAR or LAR because of the invasive nature of the procedure, and pretreatment with bronchodilators.

The allergen challenge model has been very useful for studying mechanisms of disease and efficacy of therapeutic intervention. The subjects recruited for these studies should have mild, allergic asthma, with an FEV₁ >70% of predicted normal, and not use regular medication for

the treatment of their asthma. In this case, only acute allergen-induced events are studied as these subjects do not have persistent asthma symptoms. Furthermore, studies of LAR require further selection of subjects having mild asthma who develop the required response. The LAR occurs in up to more than 50% of allergic asthmatic subjects studied, but depends on the allergen which is used for the challenge; thus inhalation of grass pollen can cause an EAR, but rarely a LAR, while house dust mite or cat allergen usually cause both. Cooperation between laboratories can be useful to carry out allergen challenge studies successfully and efficiently. It is essential, however, that methodologies are standardized and quality control procedures maintained between laboratories.

Allergen-inhalation challenge: a model to predict efficacy of asthma drugs

The allergen-induced EAR is likely of limited clinical relevance (64); when the EAR occurs clinically, it does alert the individual to the relationship between an allergen and symptoms. Occasionally, under conditions of either heavy exposure or severe allergy or both, natural exposure to inhaled allergens can cause a severe EAR; this is much more likely to occur with ingested or injected allergens. Additionally, the allergen-induced EAR can be predicted with reasonable certainty from simple tests, namely a measurement of the magnitude of specific IgE such as an allergen skin test end point, and the level of allergen-induced AHR (55). In contrast, the allergen-induced LAR, which is accompanied by increases in AHR and both metachromatic cells (65) and eosinophilic inflammation (51) more closely resembles natural occurring asthma. Consequently, we have hypothesized that the allergen challenge model, focusing particularly on the late sequelae (LAR, AHR, and inflammation) provides a useful model for evaluating efficacy or lack of efficacy of controller asthma therapies (66). Evidence for this hypothesis will be reviewed.

Asthma therapies are categorized as relievers (bronchodilator and bronchoprotector) and nonbronchodilator controllers (anti-inflammatory) (67). While this is a useful approach, the distinction is not always so clear. However, current therapies will be discussed under bronchodilator and nonbronchodilator headings. Relievers include the bronchodilators intermediate-acting β_2 -agonists and anticholinergics. The long-acting-inhaled β_2 -agonists and theophyllines fit best in this category but may have some anti-inflammatory effects.

Bronchodilators

Intermediate-acting β_2 -agonists inhibit or reverse the EAR and reverse the LAR when it is not too severe; however, they neither prevent the LAR nor any of the

associated inflammatory events (68, 69). In fact, regular use of inhaled β_2 -agonists actually enhances most aspects of the allergen-induced airway responses including the EAR (70), the LAR (71), and the allergen-induced inflammation. Anticholinergic agents have a small functional antagonist effect but do not inhibit the LAR (72, 73). Long-acting-inhaled β_2 -agonists are difficult to evaluate because of their prolonged bronchodilator and functional antagonist effects. Initial reports showing inhibition of EAR and LAR and induced AHR were thought to represent more than just the functional antagonist effect (74). However, subsequent studies have generally shown minimal inhibition of the allergen-induced inflammation and it is currently believed that these agents mainly act as functional antagonists in their inhibition of allergen-induced responses (75–81); there are some suggestions of a minor anti-inflammatory effect (82). Theophylline has a prolonged effect, which results in a most partial inhibition of the LAR (83–86) and induced AHR probably mainly because of a functional antagonist effect (87). A small study demonstrated a partial inhibition of the LAR but little effect on the induced AHR (87). There are few studies addressing airway inflammation; however, one study showed no inhibition of allergen-induced airway eosinophilia while noting a reduction in activated T cells (88).

Nonbronchodilators

The most important controller medications are ICSs. When used in single dose shortly before allergen challenge (or in the interval phase between the EAR and the LAR; 89), ICSs demonstrate no effect on the EAR with marked inhibition of the LAR (45, 69, 75, 90). Prolonged treatment improves the EAR (91–94) and probably has a greater effect on the late inflammatory sequelae.

The cromones, sodium cromoglycate, and nedocromil, when used before, but not after, allergen challenge inhibit the entire response including induced AHR (40, 43, 45, 69, 95). There are no studies addressing airway inflammation.

Leukotriene receptor antagonists are at best only slight bronchodilators and functional antagonists, for example, single-dose montelukast does not influence methacholine challenge (96). However, when these agents are used prior to allergen challenge, it appears that all aspects of the challenge including the LAR-induced AHR and induced eosinophil infiltrate can be suppressed (97–100). Comparative studies show that the suppression, while statistically and clinically significant, is not as great in magnitude as that achieved with ICSs (101, 102).

H₁ blockers have been suggested to have an antiallergic effect. This has been postulated to be a direct effect on asthma; however, indirect improvement in asthma achieved by improving upper airway symptoms is also possible. Various H₁ blockers have been examined with the allergen challenge model and, although there are some

conflicting results, for the most part, these agents, including ketotifen, probably have little, if any, effect on the late sequelae (103–109). There is at least partial inhibition of the EAR and one study has documented that the ability to administer a larger dose of allergen under the EAR protection of antihistamines results in the appearance of a LAR (110), similar to the results of administering a larger dose of allergen following EAR inhibition with inhaled β_2 -agonist (111).

Allergen challenge was used in two of the pivotal early investigations in the study of anti-IgE (omalizumab) in asthma (112, 113). Following treatment with anti-IgE, despite administration of approximately twice as much allergen compared to the placebo treatment, subjects had marked reduction in all of the late asthmatic sequelae (114).

Ineffective therapies

The value of allergen challenge, particularly the late sequelae, was examined in a proof of concept study. A clinically ineffective ICS was compared with the clinically effective corticosteroid, budesonide, in a single-dose trial involving allergen challenge. This model was able to differentiate between the clinically effective and clinically ineffective corticosteroid with regard to its effect on the allergen-induced LAR (90). This is the only study to our knowledge, designed to test the LAR model with an ineffective agent.

Other agents, which are ineffective or not very effective (alone) as maintenance asthma therapy, have been reviewed above. These included intermediate-acting β_2 -agonists, long-acting β_2 -agonists, anticholinergics, theophylline, H₁ blockers, and oral *antiallergic* agents [e.g. ketotifen (107), repirinast (114)].

In summary, the data outlined above support the hypothesis that a properly done and properly interpreted allergen challenge study can be of value to predict efficacy or lack of efficacy of asthma controller therapies. Agents which inhibit the late asthmatic sequelae including the allergen-induced LAR, allergen-induced increase in AHR, and allergen-induced inflammation are generally effective in asthma therapy (Table 1). Perhaps of more value in drug development for asthma is that compounds that have not influenced the allergen-induced late sequelae have never been subsequently proven to be effective in asthma treatment (Table 1). Thus, the test has a moderate positive predictive value, but an excellent negative predictive value.

Investigation of new agents to study the pathophysiology of allergic responses

A large number of new molecules targeting various mechanisms or pathways of the airway inflammatory process are under scrutiny and considerable efforts will be

Table 1. Prediction of clinical efficacy from inhibition of allergen-induced response

True positives	True negatives	False positives	False negatives
Conventional ICS	Esterase-sensitive steroids	Anti-CD11a	Nil
LABA	PAF antagonists	PGE ₂	
Combination ICS/LABA	Inhaled anti-LTs	PGE ₁ analogs	
SABA	Thromboxane antagonists	Antihistamines	
Anti-LT	Selectin inhibitors		
Anti-IgE			
Theophylline			

ICS, inhaled corticosteroids; LABA, long-acting β_2 -agonists; SABA, short-acting β_2 -agonists; anti-LT, antileukotrienes; PAF, platelet-activating factor; PGE, prostaglandin E.

devoted to determine if these agents may be clinically useful and improve airway inflammatory conditions such as asthma and rhinitis (115, 116). Allergy is a key mechanism leading to both the development and persistence of airway inflammation and structural changes that may result in symptomatic asthma and rhinitis (117). Methods that could rapidly determine if a new product will be useful in treating those conditions are welcome.

With standardized methods and validated outcomes, the allergen bronchoprovocation test has become such a tool which may quickly, in a very limited number of patients, provide key information on the therapeutic potential of the tested agent (90). As stated earlier in this manuscript, the test may indicate that the drug will be ineffective to treat asthma, for example, although it does not provide accurate data on the degree of therapeutic

efficacy of the agent. Nevertheless, as an initial 'screening test', it may avoid spending large amount of money and resources to evaluate its clinical usefulness.

Not only can this method help forecast clinical efficacy of the agent, but it may provide valuable information on how the agent is influencing the pathophysiology of immune responses and airway inflammation. With the new noninvasive methods of assessment of airway inflammation such as induced sputum analysis, exhaled NO, or exhaled breath condensate analysis (e.g. isoprostanes, pH, etc.) various aspects of the inflammatory response may be explored (118, 119).

Although there are still limitations to these tests, standardization procedures, and improved methods of measurement of various mediators are being developed, as well as surrogate markers of airway remodeling processes. The allergen bronchoprovocation test therefore provides a dynamic model to evaluate various clinical, physiologic, and inflammatory changes following the acute trigger of the inflammatory cascade. The newly developed LDACs may as well be useful in mimicking more closely natural exposures.

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