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Allergen-induced airway inflammation and its therapeutic intervention

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Allergen inhalation challenge has been useful for examining the mechanisms of allergen-induced airway inflammation and the associated physiological changes and for documenting the efficacy of drugs to treat asthma. Allergen inhalation by a sensitized subject results in acute bronchoconstriction, beginning within 15-30 min and lasting 1-3 hr, which can be followed by the development of a late asthmatic response. Individuals who develop both an early and late response after allergen have more marked increases in airway hyperresponsiveness, and greater increases in allergen-induced airway inflammation, particularly in airway eosinophils and basophils. All of the currently available and effective treatments for asthma modify some aspects of allergen-induced responses. These medications include short-acting and long-acting inhaled β_2-agonists, inhaled corticosteroids, cromones, methylxanthines, leukotriene inhibitors, and anti-IgE monoclonal antibody. In addition, allergen inhalation challenge has become a useful method which can, in a very limited number of patients, provide key information on the therapeutic potential of new drugs being developed to treat asthma.

Key Words: asthma; allergen; inflammation; drug development

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, which is characterized by the characteristic symptoms of dyspnea, chest tightness, cough and wheezing, and by variable airflow obstruction and airway hyperresponsiveness to a wide variety of physical and inhaled chemical stimuli. Over the past 40 yr, very effective medications have been developed to treat asthma, the most effective of which are inhaled β_2-agonists for acute symptom relief and inhaled corticosteroids (ICS) for long term management.[1] Important insights into the optimal management of asthma were made in the early 1980's, when the central role of airway inflammation was identified to be important in asthma pathogenesis, even in very mild disease.[2] This resulted in a change of focus from the relief of symptoms with frequent use of inhaled short acting β_2-agonists, to the prevention of symptoms and asthma exacerbations by the regular use of ICS. This approach is extremely effective in the majority of asthmatic patients, and in those who remain symptomatic despite ICS treatment, the combination of ICS and a long-acting inhaled β_2-agonists (such as formoterol or salmeterol) is generally sufficient to control asthma.[3]

Asthma treatment guidelines have identified that the primary goal of management is to achieve optimal asthma control.[1] Asthma control means the minimization of night time and daytime symptoms, and no activity limitation, rescue bronchodilator use or airway narrowing. The search for new and effective asthma treatments has persisted and the approaches developed to assess these new treatment approaches have changed in light of the appreciation of inflammation as central to asthma pathophysiology and the focus on asthma control as the most important treatment outcome. This review will focus on the value and limitations of allergen inhalation challenge and its associated increase in airway inflammation in the evaluation of new therapeutic options for asthma.

ACTIVITY vs EFFICACY vs EFFECTIVENESS

Drug development is a 4 phase process. Phase I is the evaluation of the new pharmacological entity for safety in normal volunteers; although this phase is sometimes also used to look for

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some evidence of activity. Phase II is when the "proof of concept" study are done, and in studies for new entities in asthma, this is usually in mildly symptomatic patients with airflow obstruction, or in a clinical model of allergic inflammation. These studies are really examining for activity of the new entity in asthmatic airways and evidence of this activity does not always translate to evidence of efficacy in asthma. Also, these Phase II studies are usually small in size and of short duration, which provides little information of the safety of the entity in asthmatic patients. Phase II is sometimes divided into Phase IIa, where the proof of concept study is done, and Phase IIb, where the entity is evaluated in small studies of more symptomatic patients, to help develop the designs for the efficacy studies. Efficacy is evaluated in Phase III studies, which are designed to meet the requirements of regulatory agencies to show both efficacy and safety in the patient population for whom the new drug is to be prescribed. These studies are large (often >1,000 patients) and long (usually 1 yr of treatment). Evidence of efficacy is required in two such studies to obtain regulatory approval of the new treatment. The requirement for the outcomes to be evaluated in these Phase III studies differs in different countries granting regulatory approval. The final phase of clinical trial development involves Phase IV studies, conducted after drug approval has been obtained. These studies are usually used to best position the drug in the marketplace and to collect additional information on safety. However, very few new drugs for asthma have been formally evaluated for effectiveness, which is the usefulness of the drug in the real world setting.

ALLERGEN INHALATION CHALLENGE

In 1873, Blackley published a monograph describing grass pollen as the cause of these seasonal symptoms of allergic rhinitis and seasonal asthma.[4] The late asthmatic response (LAR), which occurs 3 to 8 or more hours after allergen exposure, is now recognized as clinically more important than the early asthmatic response (EAR). In the 1950's, Herxheimer identified that many of patients undergoing allergen hyposensitization complained of late symptoms, and he record the development of allergen-induced LAR in a significant number of patients, more common with house dust than with pollen.[5] It is now recognized that inhaled allergens by a sensitized subject results in acute bronchoconstriction, usually beginning within 15-30 min and lasting 1-3 hr. This is called the EAR. This can then be followed by the development of an LAR beginning after the spontaneous resolution of the EAR, but which is more insidious in onset, gradually worsening over 3-12 hr, is more prolonged and often more severe than the EAR (Fig. 1).[6] Individuals who develop both an EAR and LAR after inhaled allergen (dual responders) also have more marked and prolonged increases in airway hyperresponsiveness,[7,8] and greater increases in allergen-induced airway inflammation, particularly in airway eosinophils[3,9] and basophils.[10] This clinical model of allergen inhalation challenge has been extremely useful for examining the mechanisms of allergen-induced airway inflammation and the associated physiological changes and for documenting the efficacy of drugs to treat asthma.

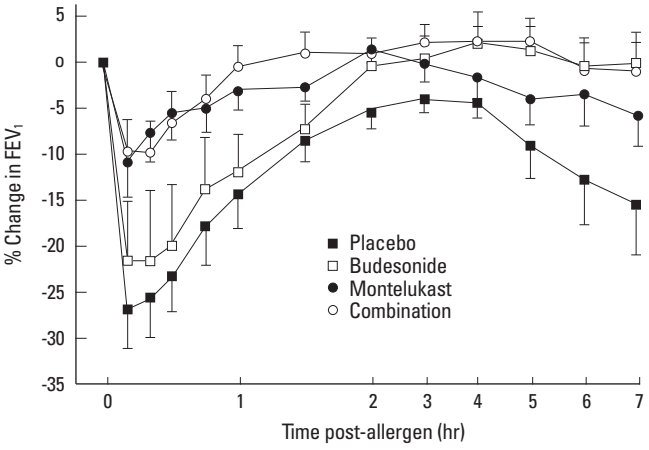

Fig. 1. The effect of therapy on the airway response to inhaled allergen; demonstrating time course of the mean (SD) decline in FEV_1 (expressed as percent of pre-challenge FEV_1 values) following allergen challenge to assess the efficacy of ten days of treatment with an inhaled corticosteroid (budesonide; 400 mcg daily) and a leukotriene antagonist (montelukast; 10 mg daily) on the early and late airway response to inhaled allergen in ten asthmatic subjects. Significant attenuation of the early response was observed with either montelukast or the combination of budesonide and montelukast when compared with placebo. Significant attenuation of the late airway response was observed by all three active treatment regimens when compared with placebo.[49]

It is now known that inhaled allergen induced their airway responses by cross-links antigen-specific immunoglobulin (Ig)E that is bound to IgE receptors (FCεRI) on mast cells resident in the airway and circulating basophils. This is followed by mast cell degranulation to release a variety of preformed mediators (e.g., histamine),[11] as well as the up regulation of eicosanoid pathways to produce newly formed mediators (e.g., leukotrienes, prostaglandins) of bronchoconstriction,[12] which also lead to increasing vascular permeability. Indeed, allergen-induced bronchoconstriction occurring during the EAR and LAR can be abolished by treatment with a combination of anti-histamine and leukotriene antagonists,[13] indicating that histamine and cysteinyl leukotrienes together are responsible for these allergen-induced effects.

PHARMACOLOGICAL MODIFICATION OF ALLERGEN-INDUCED RESPONSES

All of the currently available and effective treatments for asthma modify some aspects of allergen-induced responses. These medications include short-acting and long-acting inhaled β_2-agonists (SABAs and LABAs), inhaled corticosteroids (ICS), cromones, methylxanthines, leukotriene inhibitors, and anti-

IgE monoclonal antibody.

Inhaled β_2-agonists

Treatment with SABAs immediately before allergen inhalation inhibits or reverses the EAR and, if administered during the LAR, can partially reverse the LAR, when it is not too severe; however, they neither prevent the LAR, nor allergen-induced airway inflammation. By contrast, the regular use of SABAs has been demonstrated to enhance most aspects of the allergen-induced airway responses including the EAR,[14] the LAR[15] and the allergen-induced airway inflammation.[16] This evidence provided part of the rationale for avoiding the regular use of SABAs as monotherapy for asthma.

LABAs are difficult to evaluate because of their prolonged bronchodilator, as well as functional antagonist effects. Functional antagonism means their ability to prevent the onset of bronchoconstriction. Initial reports showing inhibition of EAR and LAR and induced AHR were thought to represent more than just the functional antagonist effect.[17] However, subsequent studies have generally shown minimal inhibition of the allergen-induced inflammation,[18] and it is currently believed that LABAs act mainly as functional antagonists in their inhibition of allergen-induced airway responses.[19-25]

Methylxanthines

Theophylline has a prolonged effect as a weak bronchodilator and a functional antagonist, which results in at most partial inhibition of the LAR.[26-30] A small study demonstrated the partial inhibition of the LAR, but little effect on the induced AHR.[30] There are few studies addressing allergen-indcued airway inflammation, however, one study showed no inhibition of allergen-induced airway eosinophilia, but a small reduction in airway activated T cells.[31]

Inhaled corticosteroids

The most important controller medications for asthma are ICS. They are known to improve all aspects of asthma control,[1] reduce eosinophilic airway inflammation[32] and reduce some components of airway remodelling.[32] ICS also have profound effects on allergen-induced airway responses. When used in single dose shortly before allergen challenge (or in the interval phase between the EAR and the LAR),[33] ICS demonstrate no effect on the EAR, but markedly inhibit the LAR.[19,34-36] Regular treatment with ICS for several weeks improves the EAR, abolishes the LAR and markedly reduces allergen-induced airway inflammation.[37-41]

Cromones

The cromones consist of two drugs, cromoglycate and nedocromil. The earliest studies of pharmacoprotection against allergen-induced responses were done with cromoglycate. These studies demonstrated that when used before, but not after, allergen challenge cromoglycate inhibits allergen-induced EAR, LAR, and allergen-induced airway hyperresponsiveness.[34,35,42-44] These data were used to support the mechanism of action of the cromones as inhibiting allergen-induced mast cell degranulation. There are no studies addressing the effects of treatment with either cromoglycate or nedocromil on allergen-induced airway inflammation.

Leukotriene inhibitors

Leukotriene inhibitors inhibit either the production of the cysteinyl leukotrienes (5-lipoxygenase inhibitors) or the action of cysteinly leukotrienes on their receptor (Cys LT_1 receptor antagonists). Treatment with leukotriene inhibitors attenuates all aspects of allergen-induced airway responses. They attenuate the EAR,[45-50] and do so to a greater extent than do ICS,[49] but are less effective than ICS in their ability to attenuate the LAR (Fig. 1), or allergen-induced AHR.[49] Leukotriene inhibitors also markedly reduced allergen-induced airway eosinophilia to a similar extent as ICS (Fig. 2).[49,50]

Various histamine antagonists (H_1 blockers) have been examined with the allergen challenge model and have been demonstrated to have small degrees of protection against the EAR, with little effect on the LAR.[50-57] Of interest, however, the combination of a leukotriene antagonist and a H_1 blocker completely abolishes both the EAR and LAR.[13] This means that the bronchoconstriction that develops after allergen inhalation is caused by the release of histamine and the cysteinyl leukotrienes, likely from mast cell activation causing the EAR and basophil activation causing the LAR.[10]

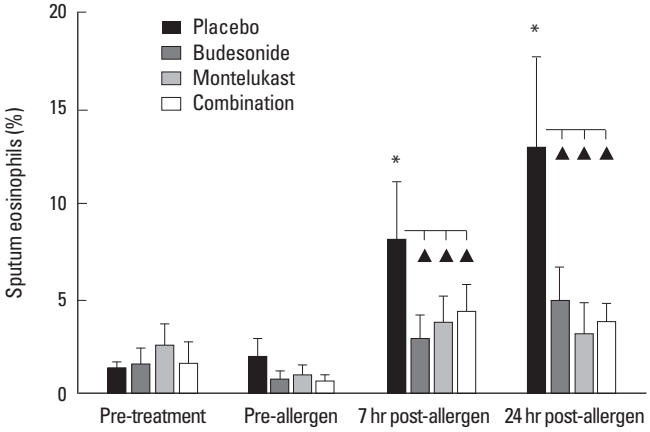

Fig. 2. The mean (SD) percentage of sputum eosinophils before and after an inhaled corticosteroid (budesonide) and a leukotriene antagonist (montelukast) before and following an allergen inhalation challenge. There was a subsequent reduction in the allergen-induced eosinophilia in the presence of all treatments. *Indicates significant difference from pre-allergen value in the same treatment group. *Filled triangles* indicate a significant difference from placebo at the same time point.[49]

Anti-IgE monoclonal antibody

Allergen inhalation challenge was used in two of the pivotal early investigations in the study of anti-IgE (omalizumab) in asthma.[58,59] Following treatment with anti-IgE, despite administration of approximately twice as much allergen compared to the placebo treatment, subjects had marked reduction in the LAR.[59]

The evaluation of ineffective therapies for asthma

The consistent demonstration of the benefits of drugs effective for asthma treatment on aspects of allergen-induced airway responses has resulted in allergen inhalation challenge becoming the most common method for the evaluation of new therapies for asthma (particularly if these are thought to have anti-inflammatory properties). Several drugs candidates have failed to modify allergen-induced responses and have also failed in larger clinical trials in asthma. Perhaps the best example of this was the evaluation of esterase-sensitive ICS. These were corticosteroids which were rapidly metabolized by esterases in the blood and therefore had the profile of being active in the airway, but potentially having no corticosteroid side effects. An interesting study was performed with one such molecule, which had been demonstrated not to work in clinical trials in asthma. This clinically ineffective esterase-sensitive ICS was compared with the clinically effective ICS, budesonide, in a single dose trial involving allergen inhalation challenge. This study demonstrated that allergen challenge was able to differentiate between the clinically effective and clinically ineffective corticosteroid with regard to its effect on the allergen-induced LAR.[36] There are other examples of candidate drugs failing to protect against allergen challenge and failing in clinical trials. These include platelet activating factor (PAF) antagonists,[60,61] thromboxane inhibitors,[62,63] VLA4 antagonists,[64] and inhaled leukotriene inhibitors.[65]

The results of these studies suggest that a well conducted and interpreted allergen challenge study can be of value to predict efficacy or lack of efficacy of asthma controller therapies. Thus, drugs which inhibit the asthmatic responses, particularly 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.

Table 1. Examples of drugs studied using allergen inhalation challenge

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 analogues	
SABA	Thromboxane antagonists	Anti-histamines	
Anti-LT	Selectin inhibitors		
Anti-IgE			
Theophylline			

*True positives are those drugs which modify the challenge and have been shown to be effective in asthma; †True negatives are drugs which did not modify allergen challenge and which have failed in larger clinical trials of asthma patients; ‡False positives are drugs which modified the challenge, but which are not useful to treat asthma; §False negatives would be drugs which did not modify the challenge, but are useful to treat asthma.
No false negatives have been identified to date.
ICS, inhaled corticosteroids; LABA, long acting β_2-agonists; SABA, short acting β_2-agonists; Anti-LT, anti-leukotrienes; PAF, platelet activating factor; PGE, prostaglandin E.

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 devoted to determine if these agents may be clinically useful and improve airway inflammatory conditions such as asthma and rhinitis.[66,67] 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.[68] 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 tool which may quickly, in a very limited number of patients, provide key information on the therapeutic potential of the tested agent.[36] 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 non-invasive 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.[69,70]

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 remodelling processes. The allergen bronchoprovocation test therefore provides a dynamic model to evaluate various clinical, physiological and inflammatory changes following the acute trigger of the inflammatory cascade. The newly developed low dose allergen challenges may as well be useful, in mimicking more closely natural exposures.

CONCLUSIONS

When embarking on the clinical development of therapeutic agents in airway disease, designing effective studies to investigate the airway response requires an understanding of the available outcomes that are clinically relevant, such as asthma exacerbations and asthma control. These should also be considered in association with the appropriate standardized physiological and biochemical markers to validate efficacy, such as markers for inflammation and airway hyperresponsiveness; the hallmarks of dysfunction in airway disease that are likely the targets of a new therapeutic agent. All outcome measures, whether chosen as primary or secondary outcomes, may have certain limitations that need to be understood before they are applied in an effort to maximize their usefulness in establishing efficacy of a therapeutic agent.

The practical and safety considerations of a chosen outcome measure, in particular those that are more invasive, should be well understood and carefully considered. When designing a clinical trial, prior studies that demonstrate the success of known therapies in airway disease should also be considered in an effort to demonstrate equivalent or superior efficacy and safety compared to existing therapies. Finally, and with no less importance, the most appropriate patient population at each stage of development of a therapeutic agent needs to be selected that reflects the broadest applicable patient population that will translate to benefits in the real world population with airway disease.

REFERENCES

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, Fitzgerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143-78.
2. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. Am Rev Respir Dis 1987;136:379-83.
3. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, Pedersen SE; GOAL Investigators Group. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med 2004;170:836-44.
4. Blackley CH. Experimental researches on the cause and nature of Catarrhus aestivus (Hay-fever or Hay-asthma). London: Balliere Tindall & Cox; 1873.
5. Herxheimer H. The late bronchial reaction in induced asthma. Int Arch Allergy Appl Immunol 1952;3:323-28.
6. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. Am Rev Respir Dis 1987;136:740-51.
7. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977;7:503-13.
8. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol 1982;70:170-7.
9. De Monchy JG, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, De Vries K. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985;131:373-6.
10. Gauvreau GM, Lee JM, Watson RM, Irani AM, Schwartz LB, O'Byrne PM. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. Am J Respir Crit Care Med 2000;161:1473-8.
11. Wood-Baker R, Finnerty JP, Holgate ST. Plasma and urinary histamine in allergen-induced early and late phase asthmatic responses. Eur Respir J 1993;6:1138-44.
12. Manning PJ, Rokach J, Malo JL, Ethier D, Cartier A, Girard Y, Charleson S, O'Byrne PM. Urinary leukotriene E4 levels during early and late asthmatic responses. J Allergy Clin Immunol 1990;86:211-20.
13. Davis BE, Illamperuma C, Gauvreau GM, Watson RM, O'Byrne PM, Deschesnes F, Boulet LP, Cockcroft DW. Single dose desloratadine and montelukast and allergen-induced late airway responses. Eur Respir J 2009;33:1302-8.
14. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. Lancet 1993;342:833-7.
15. Cockcroft DW, O'Byrne PM, Swystun VA, Bhagat R. Regular use of inhaled albuterol and the allergen-induced late asthmatic response. J Allergy Clin Immunol 1995;96:44-9.
16. Gauvreau GM, Jordana M, Watson RM, Cockroft DW, O'Byrne PM. Effect of regular inhaled albuterol on allergen-induced late responses and sputum eosinophils in asthmatic subjects. Am J Respir Crit Care Med 1997;156:1738-45.
17. Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen-induced asthma by salmeterol. Lancet 1990;336:1338-42.
18. Dente FL, Bancalari L, Bacci E, Bartoli ML, Carnevali S, Cianchetti S, Di Franco A, Giannini D, Vagaggini B, Testi R, Paggiaro PL. Effect of a single dose of salmeterol on the increase in airway eosinophils induced by allergen challenge in asthmatic subjects. Thorax 1999;54:622-4.
19. Wong BJ, Dolovich J, Ramsdale EH, O'Byrne P, Gontovnick L, Denburg JA, Hargreave FE. Formoterol compared with beclomethasone and placebo on allergen-induced asthmatic responses. Am Rev Respir Dis 1992;146:1156-60.
20. Pedersen B, Dahl R, Larsen BB, Venge P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and post-challenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X. Allergy 1993;48:377-82.
21. Weersink EJ, Aalbers R, Koeter GH, Kauffman HF, De Monchy JG, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. Am J Respir Crit Care Med

1994;150:1262-7.
22. Pizzichini MM, Kidney JC, Wong BJ, Morris MM, Efthimiadis A, Dolovich J, Hargreave FE. Effect of salmeterol compared with beclomethasone on allergen-induced asthmatic and inflammatory responses. Eur Respir J 1996;9:449-55.
23. Brusasco V, Crimi E, Gherson G, Nardelli R, Oldani V, Francucci B, Della Cioppa G, Senn S, Fabbri LM. Actions other than smooth muscle relaxation may play a role in the protective effects of formoterol on the allergen-induced late asthmatic reaction. Pulm Pharmacol Ther 2002;15:399-406.
24. Calhoun WJ, Hinton KL, Kratzenberg JJ. The effect of salmeterol on markers of airway inflammation following segmental allergen challenge. Am J Respir Crit Care Med 2001;163:881-6.
25. Dente FL, Bacci E, Bartoli ML, Cianchetti S, Di Franco A, Giannini D, Taccola M, Vagaggini B, Paggiaro PL. One week treatment with salmeterol does not prevent early and late asthmatic responses and sputum eosinophilia induced by allergen challenge in asthmatics. Pulm Pharmacol Ther 2004;17:147-53.
26. Pauwels R, Van RD, Van der SM, Johannesson N, Persson CG. The effect of theophylline and enprofylline on allergen-induced bronchoconstriction. J Allergy Clin Immunol 1985;76:583-90.
27. Crescioli S, Spinazzi A, Plebani M, Pozzani M, Mapp CE, Boschetto P, Fabbri LM. Theophylline inhibits early and late asthmatic reactions induced by allergens in asthmatic subjects. Ann Allergy 1991;66:245-51.
28. Hendeles L, Harman E, Huang D, O'Brien R, Blake K, Delafuente J. Theophylline attenuation of airway responses to allergen: comparison with cromolyn metered-dose inhaler. J Allergy Clin Immunol 1995;95:505-14.
29. Kraft M, Pak J, Borish L, Martin RJ. Theophylline's effect on neutrophil function and the late asthmatic response. J Allergy Clin Immunol 1996;98:251-7.
30. Cockcroft DW, Murdock KY, Gore BP, O'Byrne PM, Manning P. Theophylline does not inhibit allergen-induced increase in airway responsiveness to methacholine. J Allergy Clin Immunol 1989;83:913-20.
31. Jaffar ZH, Sullivan P, Page C, Costello J. Low-dose theophylline modulates T-lymphocyte activation in allergen-challenged asthmatics. Eur Respir J 1996;9:456-62.
32. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. New developments. Am J Respir Crit Care Med 1998;157:S1-53.
33. Cockcroft DW, McParland CP, O'Byrne PM, Manning P, Friend JL, Rutherford BC, Swystun VA. Beclomethasone given after the early asthmatic response inhibits the late response and the increased methacholine responsiveness and cromolyn does not. J Allergy Clin Immunol 1993;91:1163-8.
34. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses,late asthmatic responses,and increased bronchial responsiveness to histamine. J Allergy Clin Immunol 1987;79:734-40.
35. Pepys J, Davies RJ, Breslin AB, Hendrick DJ, Hutchcroft BJ. The effects of inhaled beclomethasone dipropionate (Becotide) and sodium cromoglycate on asthmatic reactions to provocation tests. Clin Allergy 1974;4:13-24.
36. Kidney JC, Boulet LP, Hargreave FE, Deschesnes F, Swystun VA, O'Byrne PM, Choudry N, Morris MM, Jennings B, Andersson N, Andreasson A, Cockcroft DW. Evaluation of single-dose inhaled corticosteroid activity with an allergen challenge model. J Allergy Clin Immunol 1997;100:65-70.
37. Pepys J. Atopy. In: Gell PGH, Coombs RRA, Lachman PJ, editors. Clinical aspects of immunology. Oxford: Blackwell Scientific Publications; 1975. p. 877-902.
38. Burge PS, Efthimiou J, Turner-Warwick M, Nelmes PT. Double-blind trials of inhaled beclomethasone diproprionate and fluocortin butyl ester in allergen-induced immediate and late asthmatic reactions. Clin Allergy 1982;12:523-31.
39. De Baets FM, Goeteyn M, Kerrebijn KF. The effect of two months of treatment with inhaled budesonide on bronchial responsiveness to histamine and house-dust mite antigen in asthmatic children. Am Rev Respir Dis 1990;142:581-6.
40. Swystun VA, Bhagat R, Kalra S, Jennings B, Cockcroft DW. Comparison of 3 different doses of budesonide and placebo on the early asthmatic response to inhaled allergen. J Allergy Clin Immunol 1998;102:363-7.
41. Wong CS, Wahedna I, Pavord ID, Tattersfield AE. Effect of regular terbutaline and budesonide on bronchial reactivity to allergen challenge. Am J Respir Crit Care Med 1994;150:1268-73.
42. Booij-Noord H, Orie NG, De VK. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971;48:344-54.
43. Pepys J, Hargreave FE, Chan M, McCarthy DS. Inhibitory effects of disodium cromoglycate on allergen-inhalation tests. Lancet 1968;2:134-7.
44. Dahl R, Pedersen B. Influence of nedocromil sodium on the dual asthmatic reaction after allergen challenge: a double-blind, placebo-controlled study. Eur J Respir Dis 1986;69:263-5.
45. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. Lancet 1991;337:690-4.
46. Dahlen B, Zetterstrom O, Bjorck T, Dahlen SE. The leukotriene-antagonist ICI-204,219 inhibits the early airway reaction to cumulative bronchial challenge with allergen in atopic asthmatics. Eur Respir J 1994;7:324-31.
47. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. J Allergy Clin Immunol 1998;102:177-83.
48. Diamant Z, Grootendorst DC, Veselic-Charvat M, Timmers MC, De Smet M, Leff JA, Seidenberg BC, Zwinderman AH, Peszek I, Sterk PJ. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. Clin Exp Allergy 1999;29:42-51.
49. Leigh R, Vethanayagam D, Yoshida M, Watson RM, Rerecich T, Inman MD, O'Byrne PM. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. Am J Respir Crit Care Med 2002;166:1212-7.
50. Palmqvist M, Bruce C, Sjostrand M, Arvidsson P, Lotvall J. Differential effects of fluticasone and montelukast on allergen-induced asthma. Allergy 2005;60:65-70.
51. Nakazawa T, Toyoda T, Furukawa M, Taya T, Kobayashi S. Inhibitory effects of various drugs on dual asthmatic responses in wheat flour-sensitive subjects. J Allergy Clin Immunol 1976;58:1-9.
52. Adachi M, Kobayashi H, Aoki N, Iijima M, Kokubu F, Furuya A,

Takahashi T. A comparison of the inhibitory effects of ketotifen and disodium cromoglycate on bronchial responses to house dust, with special reference to the late asthmatic response. Pharmatherapeutica 1984;4:36-42.
53. Rafferty P, Ng WH, Phillips G, Clough J, Church MK, Aurich R, Ollier S, Holgate ST. The inhibitory actions of azelastine hydrochloride on the early and late bronchoconstrictor responses to inhaled allergen in atopic asthma. J Allergy Clin Immunol 1989;84:649-57.
54. Hamid M, Rafferty P, Holgate ST. The inhibitory effect of terfenadine and flurbiprofen on early and late-phase bronchoconstriction following allergen challenge in atopic asthma. Clin Exp Allergy 1990;20:261-7.
55. Cockcroft DW, Keshmiri M, Murdock KY, Gore BC. Allergen-induced increase in airway responsiveness is not inhibited by acute treatment with ketotifen or clemastine. Ann Allergy 1992;68:245-50.
56. Twentyman OP, Ollier S, Holgate ST. The effect of H1-receptor blockade on the development of early- and late-phase bronchoconstriction and increased bronchial responsiveness in allergen-induced asthma. J Allergy Clin Immunol 1993;91:1169-78.
57. Wasserfallen JB, Leuenberger P, Pecoud A. Effect of cetirizine, a new H1 antihistamine, on the early and late allergic reactions in a bronchial provocation test with allergen. J Allergy Clin Immunol 1993;91:1189-97.
58. Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, DeVault A, Fick RB Jr, Cockcroft DW. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997;155:1835-40.
59. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB Jr, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997;155:1828-34.
60. Freitag A, Watson RM, Matsos G, Eastwood C, O'Byrne PM. Effect of a platelet activating factor antagonist, WEB 2086, on allergen induced asthmatic responses. Thorax 1993;48:594-8.
61. Evans DJ, Barnes PJ, Cluzel M, O'Connor BJ. Effects of a potent platelet-activating factor antagonist, SR27417A, on allergen-induced asthmatic responses. Am J Respir Crit Care Med 1997;156:11-6.
62. Manning PJ, Stevens WH, Cockcroft DW, O'Byrne PM. The role of thromboxane in allergen-induced asthmatic responses. Eur Respir J 1991;4:667-72.
63. Beasley RC, Featherstone RL, Church MK, Rafferty P, Varley JG, Harris A, Robinson C, Holgate ST. Effect of a thromboxane receptor antagonist on PGD2- and allergen-induced bronchoconstriction. J Appl Physiol 1989;66:1685-93.
64. Norris V, Choong L, Tran D, Corden Z, Boyce M, Arshad H, Holgate S, O'Connor B, Millet S, Miller B, Rohatagi S, Kirkesseli S. Effect of IVL745, a VLA-4 antagonist, on allergen-induced bronchoconstriction in patients with asthma. J Allergy Clin Immunol 2005;116:761-7.
65. O'Shaughnessy KM, Taylor IK, O'Connor B, O'Connell F, Thomson H, Dollery CT. Potent leukotriene D4 receptor antagonist ICI 204,219 given by the inhaled route inhibits the early but not the late phase of allergen-induced bronchoconstriction. Am Rev Respir Dis 1993;147:1431-5.
66. Barnes PJ. New therapies for asthma. Trends Mol Med 2006;12:515-20.
67. Nagai H, Teramachi H, Tuchiya T. Recent advances in the development of anti-allergic drugs. Allergol Int 2006;55:35-42.
68. Holt PG. Key factors in the development of asthma: atopy. Am J Respir Crit Care Med 2000;161:S172-5.
69. Kips JC, Inman MD, Jayaram L, Bel EH, Parameswaran K, Pizzichini MM, Pavord ID, Djukanovic R, Hargreave FE, Sterk PJ. The use of induced sputum in clinical trials. Eur Respir J Suppl 2002;37:47s-50s.
70. Kharitonov SA, Barnes PJ. Exhaled biomarkers. Chest 2006;130:1541-6.