

EDITORIAL

Bronchial allergen challenge: a model for chronic allergic asthma?

Allergen bronchial provocation tests are used extensively in research to study the pathophysiological effect of exposure to allergen on airways and to evaluate the effectiveness of pharmacological agents. They represent a major undertaking with a predictable degree of patient discomfort and a significant risk of severe bronchospasm. It is therefore essential that the results are reproducible and comparable, and meaningful data are obtained. A number of variables may influence the outcome of the test. The quality and dose of allergen extract, methodology used for allergen inhalation, measurement of bronchoconstriction and interpretation of data can all influence the result. It is crucial that sources of variability are kept to a minimum and different methods commonly used yield reproducible and comparable result.

Allergenic extracts are complicated mixtures of antigenic components with a number of detectable antigens. Ideally, standardized extracts should be used for allergen challenge. The type of nebulizer used to generate aerosol determines the particle size and hence may influence the deposition at the receptor site [1]. The type of diluent used to dilute lyophilized allergen extract and storage conditions may affect the allergenicity of the extract.

Protocols for the delivery of allergen extract to the airways vary, but two methods are commonly used. In the tidal breathing method [2], aerosol is generated continuously by a nebulizer and inhaled for a fixed timed period or number of breaths. Incremental concentrations of allergen are delivered until a minimum predetermined drop (usually 20%) in FEV_1 is achieved. The concentration at which this drop is attained is termed PC_{20} allergen. The initial dilution could be arbitrarily chosen starting with a very dilute solution or calculated from skin end-point titration [3]. A disadvantage of the tidal breathing method is that the actual dose delivered to the mouth cannot be estimated accurately.

A dosimeter can be used in conjunction with a nebulizer. In this method, aerosolized allergen is delivered through a breath-actuated dosimeter, which can be set to deliver the required dose for each number of breaths. Incremental concentrations of allergen extract may be used as in the tidal breathing method [4]. The dose at which a 20% drop in FEV_1 is achieved is termed PD_{20} allergen, and the cumulative dose given during challenge can be estimated (cumulative PD_{20} allergen). The two methods are highly comparable [5]. Which method is used is not critical so long as consistency is maintained to keep between-subject

and within-subject variation to a minimum. The use of a dosimeter is preferred as the dose of allergen can be more accurately estimated.

Airway response to an inhaled allergen is seen in only those asthmatics with specific sensitization to that allergen. Herxheimer, in 1952, formally described two distinct components of the asthmatic response [6]. Following allergen inhalation, bronchoconstriction occurs immediately and gradually improves over the next 2 h, the early asthmatic response (EAR). This is primarily due to smooth muscle contraction and mediated by histamine, prostaglandins and leukotrienes. In nearly 50% of subjects a second response is seen 2–3 h after challenge and may last up to 24 h, the late asthmatic response (LAR) [7]. Traditionally, a decrease in FEV_1 of >15% between 2 and 12 h after challenge is considered diagnostic of LAR.

The development of early and late asthmatic response is primarily related to the dose of allergen delivered [8]. Another method of assessing bronchial responsiveness to allergen is to deliver a constant dose of allergen, while comparing the resulting bronchoconstriction as measured by spirometry or airway resistance. This has also been shown to be reproducible and comparable with the traditional incremental methods [9,10]. This constant dose can be arbitrarily chosen [10], calculated from non-specific bronchial responsiveness and allergen sensitivity [11], or predefined as a cumulative dose of allergen (PD_{20}) from a previous challenge. For studies requiring repeated bronchial challenges, for example, before and after therapeutic intervention, it is convenient to give the cumulative dose (PD_{20}) as a bolus on subsequent challenges and compare the airway response. Taylor *et al.* in this issue of the Journal [12] compared incremental and bolus allergen challenge and found these to be highly correlated. The magnitude of the early and late asthmatic response, measured as mean maximum fall in FEV_1 or change in FEV_1 over time (area under the curve), were very similar with the two methods. The results reassure investigators of the validity of the data produced when using these methods.

It is also important that the resulting bronchoconstriction is measured accurately. Measurement of specific airway resistance (sRaw) and determination of FEV_1 are comparable [13]. Patient-administered peak expiratory flow is able to detect LAR but diurnal variation may interfere with accurate assessment of the response [14]. FEV_1 is a simple and reliable parameter to measure changes in

airway calibre. Both the mean maximum fall and area under the curve throughout the duration of response, may be used and give comparable results [13]. Another source of variability could be the asthma treatment being taken by the patient. β_2 -agonists and cromoglycate may inhibit EAR, whereas steroids and cromoglycate may suppress LAR and ensuing non-specific bronchial hyperresponsiveness [15].

During LAR, bronchoconstriction occurs as a result of inflammatory cellular (eosinophils, lymphocytes, basophils and neutrophils) influx, mucosal oedema and smooth muscle contraction. During LAR, an increase in eosinophil number and activation is observed [16]. Once activated, eosinophils release toxic products, including eosinophil cationic protein (ECP), which is able to damage bronchial structures and increase bronchial hyperresponsiveness [16–19]. Allergen challenge also induces an increased absolute number of IL-5-positive peripheral blood eosinophils and lymphocytes [20]. This eosinophilic inflammation in allergen-induced LAR is mediated by TH2 lymphocytes [21]. Non-specific airway responsiveness, as assessed by histamine or methacholine challenge, is increased for a week or more in those who develop LAR [22,23]. An increase in airway responsiveness after allergen challenge may precede the development of LAR [24].

Bronchial challenge is highly reproducible (one doubling dose of PD_{20}) within the same subject [25]. However, more than 100-fold variation in PD_{20} is seen between subjects. So what determines airway response to allergen? The degree of allergen sensitization (assessed by skin end-point titration or level of specific immunoglobulin E) and non-specific bronchial hyperresponsiveness, can predict PD_{20} with some degree of accuracy [11,26–28]. However, some believe that the degree of non-specific bronchial hyperresponsiveness is the main determinant of EAR and the degree of allergic sensitivity does not contribute to its prediction [29].

There is considerable debate as to what predicts the development of LAR [21,30–32]. Severity of asthma, degree of sensitivity to allergen (skin test, RAST and PD_{20} allergen), intensity of EAR and degree of non-specific bronchial reactivity have all been proposed. Machado and Stalenheim suggested that the occurrence of LAR depends on the dose of allergen administered [31]. They rechallenged nine patients with an isolated immediate response on a previous challenge with a higher dose of allergen, and demonstrated a late reaction in all. The development of LAR after allergen inhalation may be predicted by allergen-specific responsiveness of peripheral T lymphocytes *in vitro* [21].

The allergen challenge induces a state of airway hyperresponsiveness and inflammation not dissimilar to an asthmatic airway [32]. Allergen challenge, in the form of LAR, induces reversible bronchoconstriction, airway inflammation and bronchial hyperresponsiveness — three important

characteristics of asthma. However, its relevance to clinical asthma has been questioned [33]. The doses required to produce an early and late asthmatic response are not often encountered in daily life. Chronic allergic asthma can indeed be simulated to a mild but continuous form of isolated LAR (without preceding EAR), induced by natural exposure to low doses of allergens. Ihre *et al.* [34] suggested that low dose allergen challenge tends to produce an isolated late reaction supporting this hypothesis. Repeated exposure to allergen during the grass pollen season induces bronchial hyperresponsiveness and an inflammatory response involving T cells, mast cells and eosinophils [35].

Low doses of house dust mite allergen, equivalent to estimated natural exposure, inhaled in experimental conditions over a period of 4 weeks, increased bronchial responsiveness and reduced pulmonary function in mild asthmatics [36]. Isolated LAR and increased bronchial responsiveness was reported after repeated exposure to low doses of allergen over a period of 5–7 days [37]. An increase in bronchial reactivity, in the absence of clinical symptoms or signs of activation of inflammatory cells in peripheral blood, following repeated low dose allergen exposure over a week, was reported by Roquet *et al.* [38]. More research is needed to standardize the low dose repeated allergen challenge procedure and prove its usefulness in preference to traditional methods.

In summary, bronchial allergen challenge is a measure of airway responsiveness and reactivity to allergen, and in addition, proves target organ sensitivity. The development of early and late asthmatic responses depends on the severity of asthma, the degree of allergen sensitization and the dose of allergen. All methods give reliable and comparable data as long as standardized procedures are employed. The use of a dosimeter is preferred as it allows accurate estimation of the dose of allergen. The use of bolus allergen challenge for repeated tests on the same subject seems to be an acceptable procedure. The jury is still out as to the usefulness of low dose repeated allergen exposure method of bronchial provocation.

References

- 1 Ryan G, Dolovich MB, Obminski G *et al.* Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J Allergy Clin Immunol* 1981; 67:156–61.
- 2 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7:235.
- 3 Cartier A, Thompson NC, Frith PA. Allergen induced increased in bronchial responsiveness to histamine: relationship to the late asthmatic response and the change in airway calibre. *J Allergy Clin Immunol* 1982; 70:170–7.
- 4 Chai H, Farr RS, Froehlich LA *et al.* Standardisation of

- bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:323–7.
- 5 Frolund L, Svendsen UG, Nielsen NH, Weeke B, Madsen F. Bronchial allergen challenge: comparison between two different methods of provocation. *Clin Allergy* 1987; 17:439–48.
 - 6 Herxheimer H. The late bronchial reaction in induced asthma. *Int Arch Allergy* 1952; 3:323–8.
 - 7 Brooij-noord H, de Vries K, Sluiter HJ, Orie NGM. Late bronchial obstructive reaction to experimental inhalation of house-dust extract. *Clin Allergy* 1972; 2:43–61.
 - 8 Frolund L, Madsen F, Scharling B, Heinig JH, Gerner Svendsen U. Bronchial allergen challenge: dose versus concentration. *Clin Exp Allergy* 1992; 22:219–25.
 - 9 Inman MD, Watson R, Cockcroft DW, Wong BJ, Hargreave FE, O'Byrne PM. Reproducibility of allergen-induced early and late asthmatic responses. *J Allergy Clin Immunol* 1995; 95:1191–5.
 - 10 Tamura G, Mue S, Ishihara T, Takishima T. The single exposure method for inhalation challenge with allergen. *J Allergy Clin Immunol* 1985; 75:47–54.
 - 11 Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987; 135:264–7.
 - 12 Taylor DA, Harris JG, O'Connor BJ. Comparison of incremental and bolus dose inhaled allergen in asthmatic patients. *Clin Exp Allergy* 1999; 30:56–63.
 - 13 Frolund L. Bronchial allergen challenge: methodological studies, measurement of airway response. *Allergy* 1996; 51 (Suppl. 29):22–9.
 - 14 Giannini D, Paggiaro PL, Moscato G et al. Comparison between peak expiratory flow and forced expiratory Volume in one second (FEV₁) during bronchoconstriction induced by different stimuli. *J Asthma* 1997; 34:105–11.
 - 15 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.
 - 16 Durham SR, Loegering DA, Dunnette S, Gleich GJ, Kay AB. Blood eosinophils and eosinophil-derived proteins in allergic asthma. *J Allergy Clin Immunol* 1989; 84:931–6.
 - 17 Oddera S, Silvestri M, Penna R, Galeazzi G, Crimi E, Rossi GA. Airway eosinophilic inflammation and bronchial hyperresponsiveness after allergen inhalation challenge in asthma. *Lung* 1998; 176:237–47.
 - 18 Brusasco V, Crimi E, Gianiorio P, Lantero S, Rossi GA. Allergen-induced increase in airway responsiveness and inflammation in mild asthma. *J Appl Physiol* 1990; 69:2209–14.
 - 19 Woolley KL, Adelroth E, Woolley MJ, Ellis R, Jordana M, O'Byrne PM. Effects of allergen challenge on eosinophils, eosinophil cationic protein, and granulocyte-macrophage colony-stimulating factor in mild asthma. *Am J Respir Crit Care Med* 1995; 151:1915–24.
 - 20 Hallden G, Hellman C, Gronneberg R, Lundahl J. Increased levels of IL-5 positive peripheral blood eosinophils and lymphocytes in mild asthmatics after allergen inhalation provocation. *Clin Exp Allergy* 1999; 29:595–603.
 - 21 van der Veen MJ, Van Neerven RJ, De Jong EC, Aalberse RC, Jansen HM, van der Zee JS. The late asthmatic response is associated with baseline allergen-specific proliferative responsiveness of peripheral T lymphocytes in vitro and serum interleukin-5. *Clin Exp Allergy* 1999; 29:217–27.
 - 22 Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. *Thorax* 1987; 42:302–8.
 - 23 Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977; 7:503–13.
 - 24 Durham SR, Craddock CF, Cookson WO, Benson MK. Increases in airway responsiveness to histamine precede allergen-induced late asthmatic responses. *J Allergy Clin Immunol* 1988; 82:764–70.
 - 25 Kopferschmitt-Kubler MC, Bigot H, Pauli G. Allergen bronchial challenge tests: Variability and reproducibility of the early response. *J Allergy Clin Immunol* 1987; 80:730–40.
 - 26 Cockcroft DW, Ruffin RE, Frith PA et al. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 1979; 120:1053–8.
 - 27 Haugaard L, Iversen M, Dahl R. Predictors of early- and late-phase reactions to bronchial allergen challenge. *Allergy* 1997; 52:999–1004.
 - 28 Nathan RA, Kinsman RA, Spector SL, Horton DJ. Relationship between airways response to allergens and nonspecific bronchial reactivity. *J Allergy Clin Immunol* 1979; 64:491–9.
 - 29 van der Veen MJ, Lopuhaa CE, Aalberse RC, Jansen HM, van der Zee JS. Bronchial allergen challenge with isolated major allergens of *Dermatophagoides pteronyssinus*: the role of patient characteristics in the early asthmatic response. *J Allergy Clin Immunol* 1998; 102:24–31.
 - 30 MacIntyre D, Boyd G. Factors influencing the occurrence of a late reaction to allergen challenge in atopic asthmatics. *Clin Allergy* 1984; 14:311–7.
 - 31 Machado L, Stalenheim G. Factors influencing the occurrence of late bronchial reactions after allergen challenge 1990; 45:268–74.
 - 32 O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987; 136:740–51.
 - 33 Muller B, Leick C, Smith R, Suelzer M, Richerson H. Comparison of specific and non-specific bronchoprovocation in subjects with asthma, rhinitis and healthy subjects. *J Allergy Clin Immunol* 1993; 91:758–72.
 - 34 Ihre E, Axelsson IGK, Zetterstrom O. Late asthmatic reaction and bronchial variability after challenge with low doses of allergen. *Clin Allergy* 1988; 18:557–67.
 - 35 Djukanovic R, Feather I, Christina G et al. Effect of natural allergen exposure during the grass pollen season on airways inflammatory cells and asthma symptoms. *Thorax* 1996; 51:575–81.
 - 36 Arshad SH, Hamilton RG, Adkinson NF Jr. Repeated aerosol exposure to small doses of allergen — a model for chronic allergic asthma. *Am J Respir Crit Care Med* 1998; 157:1900–6.

- 37 Ihre E, Zetterstrom O. Increase in non-specific bronchial responsiveness after repeated inhalation of low doses of allergen. *Clin Exp Allergy* 1993; 23:298–305.
- 38 Roquet A, Lagging E, Ihre E et al. No signs of activity markers in peripheral blood despite increased bronchial reactivity after repeated low-dose allergen exposure. *APMIS* 1998; 106:293–9.

S. H. ARSHAD
University Medicine
Level D, Centre Block (801)
Southampton General Hospital
Tremona Road Southampton SO30 3RN
UK