Understanding allergic asthma from allergen inhalation tests

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The allergen challenge has evolved, in less than 150 years, from a crude tool used to document the etiology of allergen-induced disease to a well-controlled tool used today to investigate the pathophysiology and pharmacotherapy of asthma. Highlights of the authors' involvement with the allergen challenge include confirmation of the immunoglobulin E-dependence of the late asthmatic response, importance of (nonallergic) airway hyper-responsiveness as a determinant of the airway response to allergen, identification of allergen-induced increase in airway hyper-responsiveness, documentation of beta₂-agonist-induced increase in airway response to allergen (including eosinophilic inflammation), advances in understanding the pathophysiology and kinetics of allergen-induced airway responses, and development of a multicentre clinical trial group devoted to using the allergen challenge for investigating promising new therapeutic strategies for asthma.

Key Words: Airway responsiveness; Allergen challenge; Asthma; Eosinophils

Airway challenge with allergen was first used in the latter half of the 19th century as a means to document the etiology (at that time controversial) of what we now recognize as allergic rhinitis and allergic asthma (1). Allergen challenge has evolved, particularly over the past 40 years, to a carefully controlled procedure, which has provided invaluable insight into the mechanisms and pathogenesis of asthma, and also evolved as a tool to investigate both new and old pharmacological agents. The present report reviews the history of the allergen challenge and summarizes the important advances that have been made using this model.

HISTORY

It is less than 200 years since Bostock (2) provided the first good clinical description of what we now recognize as seasonal allergic rhinitis and asthma. This condition, reported to be quite uncommon at that time, was known as summer catarrh or rose catarrh, roses likely being a more visible but coincidental marker of the grass pollen season. The terms hay fever and hay asthma appeared in the English language circa 1828 (3) because of the common relationship of these symptoms to the haying (and grass) season. Controversy existed as to the cause

Comprendre l'asthme allergique à partir des tests d'inhalation des allergènes

Les tests de provocation allergénique ont évolué en moins de 150 ans, passant d'outils sans finesse utilisés pour documenter l'étiologie de maladies provoquées par les allergènes, à des instruments bien contrôlés, désormais utilisés pour étudier la physiopathologie et la pharmacothérapie de l'asthme. Les points saillants des travaux réalisés par les auteurs sur les tests de provocation allergénique incluent : la confirmation de la dépendance de la réponse asthmatic tardive à l'immunoglobuline E, l'importance de l'hyperréactivité bronchique (non allergique) à titre de facteur déterminant dans la réponse des voies respiratoires aux allergènes, l'identification de l'augmentation de l'hyperréactivité bronchique induite par les allergènes, la documentation de l'exacerbation de la réactivité des voies respiratoires aux allergènes induite par les bêta₂-agonistes (y compris l'inflammation à éosinophiles), les avancées dans la compréhension de la physiopathologie et de la cinétique des réactions respiratoires induites allergéniques et la mise sur pied d'un groupe pour la conduite d'essais cliniques multicentriques consacrés à l'utilisation des tests de provocation allergéniques dans la recherche sur de nouvelles stratégies thérapeutiques prometteuses pour l'asthme.

of the symptoms; some experts attributed the symptoms to emanations from the hay, while others felt that this was coincidence and the true cause was related to a combination of heat, sunshine, humidity and exercise (3). In 1873, Charles Blackley (1) published his classic monograph, documenting pollen – grass pollen in particular – as the cause of these seasonal symptoms. Blackley does refer to some published cases of nasal symptoms induced by the application of whole pollen grains; however, Blackley's treatise is widely regarded as the first substantial publication using allergen challenge. Subsequently, numerous other allergens (pollen, mammals, molds, arthropods, etc) have been identified and allergic disease has become strikingly more prevalent, being described as a postindustrial revolution epidemic (4).

In the early part of the 20th century, before approximately 50 years ago, there are scattered published reports regarding allergen challenges (5-14). Challenges were generally performed with nebulized solutions of allergen. The challenges focused primarily on the immediate or so-called early asthmatic response (EAR). The end points were, by today's standards, very insensitive, and included signs and symptoms of asthma, as well as insensitive measures of lung function such

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as maximum breathing capacity and vital capacity. Challenges were used both as a diagnostic tool (14) and as a means of allergen hyposensitization (10).

The late asthmatic response (LAR), which occurs 3 h to 8 h or more after allergen exposure, is now recognized as clinically more important than the EAR (15). There is an excellent clinical description of an allergen-induced LAR following accidental high-dose grass pollen exposure in Blackley's monograph (1). The next reports of LARs did not occur until the early 1950s. In Herxheimer's experiments (8-10,13), primarily involving hyposensitization, he noted that many of his patients complained of late symptoms, but with one exception, objective data were not obtained. Herxheimer surmised that most of these represented continuation of the EAR, often with interval improvement produced by the bronchodilator administered for its treatment. He did, however, record isolated symptomatic LARs in a significant number of patients, more common with house dust than with pollen (13).

In the 1940s in France, Tiffeneau and Pinelli (16) used new technology to measure (for the first time) expiratory flow rates. They described what in English has become known as the forced expiratory volume in 1 s (17), and by the late 1950s, this had become the standard lung function test used to assess airflow obstruction and to monitor bronchoprovocation, including allergen challenges (18). Using his new technology, the biphasic nature of the airway response to allergen (EAR, spontaneous resolution and LAR) was recognized (19). The allergen challenge model was used, particularly in the Netherlands (20-23) and in the United Kingdom (24-27), to document effectiveness of asthma drugs. The immunopathogenesis of the LAR was hotly debated. Professor Pepys (25) in the United Kingdom, who was researching both asthma and allergic alveolitis, as well as allergic bronchopulmonary aspergillosis (which may combine some features of both asthma and alveolitis), strongly suggested that the LAR was a type III or immune complex hypersensitivity response, whereas the Dutch argued that evidence favoured a type I or immunoglobulin E (IgE)-mediated (IgE being identified in 1967 [28]) response (23). Dr Hargreave's training and experience in professor Pepys' laboratory led to further studies in this area. These include several first-time important observations; highlights over the past 35 years are reviewed below.

EARLY STUDIES

IgE-dependence of the LAR

The first allergen challenge study published from the Hamilton, Ontario, group is a study documenting ragweed pollen as a common cause of late responses in both the airway and the skin (29). The available immunological data in these subjects failed to support type III hypersensitivity while favouring the likelihood of the IgE-dependence of the LAR. The initial studies confirming the IgE-dependence of the late cutaneous allergic response (30) and the LAR (31) were also performed in Hamilton using polyclonal antihuman IgE, which degranulates mast cells and mimics IgE-mediated allergic disease.

AHR as a determinant of airway response to allergen

Lack of (good) correlation between cutaneous sensitivity to allergen and airway sensitivity to allergen suggested that an additional factor was involved with the shock organ, in this

case, the lung (32). Tiffeneau and Pinelli (33) in France and Zuidema (34) in the Netherlands suggested that this factor may well be the degree of AHR to histamine or methacholine and that, with knowledge of the degree of allergy and the level of AHR, it should be possible to predict airway response to allergen. Zuidema (34) produced some preliminary results with house dust mite challenges to support this hypothesis. Killian et al (35) in 1976 were the first to provide objective documentation of the importance of AHR in determining the EAR to allergen. This was supported by a larger study by Cockcroft et al (36), presented in a manner that was conceptually easier for audiences to comprehend. Indeed, the authors found that this relationship was useful in that the amount of allergen required to produce an EAR can be predicted with reasonable certainty by the level of allergen-specific IgE (assessed by skin test end point, for example) and the magnitude of AHR (assessed by histamine or methacholine challenge) (37).

Allergen-induced AHR

The demonstration of allergen-induced AHR is arguably the most important single step forward in understanding the pathogenesis of asthma. Before its documentation, it appears that AHR, which is a consistent and indeed a defining feature of asthma, had been regarded as a constant rather than a variable feature. Following on the observations of Altounyan (38), who demonstrated seasonal increases in AHR to histamine, Cockcroft et al (39) in 1977 demonstrated that airway responsiveness to both histamine and methacholine increased in some subjects following allergen challenge (39). This increase was occasionally marked and long lasting, and subsequent studies confirmed the relationship with both the allergen-induced LAR (40) and the seasonal-induced increases in AHR (41). This provided the physiological background for the important concept differentiating asthma stimuli into inducers, ie, those which cause asthma, and inciters, ie, those which induce bronchoconstriction and symptoms in subjects who already have asthma (42). These data also led logically to the re-recognition of airway inflammation as a primary factor in the pathogenesis of (allergen-induced) asthma; these data were supported by observations that the LAR correlated with allergen-induced increase in airway eosinophilia both by bronchoalveolar lavage (43) and by sputum analyses (44), and that AHR itself correlated positively with bronchoalveolar lavage eosinophils and metachromatic cells, a feature identified for the first time in Hamilton (45). Allergen-induced AHR and allergen-induced airway eosinophilia (with or without other markers of airway inflammation) have now become components of most standardized allergen challenge protocols.

Inhaled beta₂-agonists and airway response to allergen

While investigating tolerance to the bronchoprotective effect of inhaled beta₂-agonists, Cockcroft et al (46) made the unexpected but important observation that the regular use of inhaled beta₂-agonists increased airway response to allergen significantly and consistently. Follow-up studies in both Saskatoon, Saskatchewan, and Hamilton documented that this extended to all areas of the allergen-induced response, including the EAR (46,47), the LAR (48,49) and the allergen-induced airway inflammation (49), probably as a result of regularly inhaled beta₂-agonists causing mast cell

dysfunction and increased mediator release (50). These important data provide a plausible mechanism behind the observations that regularly inhaled beta-agonists result in worsened asthma control (51).

Mechanisms of allergen-induced airway responses

Allergen inhalation challenge has been a useful clinical model to study the mechanisms of allergen-induced airway responses and airway inflammation. The fact that sensitized individuals challenged with inhaled allergens can develop either isolated early responses or dual responses allowed the evaluation of the importance (previously assumed) of progressively worsening airway inflammation in causing the LAR. These studies were greatly helped by the development of methodology for sputum induction and characterization of sputum inflammatory cells, also in Hamilton (52,53), which allowed for a non-invasive method of following the appearance of inflammatory cells into the airway lumen. Studies (54) conducted in Hamilton demonstrated that while individuals with isolated EARs developed an eosinophilic and basophilic airway inflammatory response, this was much greater in those with LARs. Also, the maximum influx of basophils was earlier (7 h after challenge) than for eosinophils (24 h after challenge) (55). Subsequently, studies of allergen-induced eosinophilopoiesis in bone marrow demonstrated that allergen inhalation caused the upregulation of the interleukin (IL)-5 receptor on bone marrow eosinophil progenitors (56), which was caused by cysteinyl leukotriene release following allergen inhalation (57), and increased production of IL-5 in the bone marrow (58), resulting in increased production of eosinophils (59).

Studies (60) examining the mechanisms by which allergen-induced airway inflammation is attenuated in isolated early responders suggested that contrasting profiles of the anti-inflammatory cytokines, interferon-gamma and IL-12 released from T-cells may be responsible for different time courses of allergen-induced airway responses between isolated early and dual responders. Finally, the trafficking of antigen presenting cells (dendritic cells) from the blood after allergen inhalation

was studied. The proinflammatory, myeloid dendritic cells decrease in the peripheral blood within 4 h after allergen inhalation (61) and appear in the airways after 6 h (62). This trafficking is, once again, dependent on cysteinyl leukotriene release after allergen inhalation (63).

Asthma drug evaluation

The allergen-induced LAR and AHR are considered to be the result of eosinophilic airway inflammation. Drugs that inhibit eosinophilic inflammation are effective asthma medications because they also improve AHR and airflow limitation. Consequently, the allergen challenge model has been useful to explore the efficacy of asthma drugs. Using the standardized reproducible methods outlined above, inhibition of the allergen-induced late sequelae (LAR, AHR and eosinophils) is hypothesized to predict therapeutic value in asthma. Examples include studies showing marked inhibition of the LAR (64-67), AHR (64-67) and eosinophil influx (67) by clinically effective inhaled corticosteroids, while there was no such inhibition when a clinically ineffective inhaled corticosteroid was given (65). Suppression of late sequelae has also been observed with other effective agents, including sodium cromoglycate (64), montelukast sodium (67) and omalizumab (68).

We have successfully pooled our resources with multicentre allergen challenge trials investigating new molecules (69-72), allowing studies to be done in a limited number of subjects in a timely manner. The Canadian Networks of Centres of Excellence, AllerGen NCE Inc, has established a clinical investigator collaborative group (PM O'Byrne and LP Boulet Theme leaders), which has provided a formalized standard multicentre allergen challenge group and has successfully participated in several studies of novel molecules in the past two years. We believe this to be an effective screening test, which may avoid unnecessary large and costly trials.

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