

BRONCHIAL ALLERGEN CHALLENGES IN ASTHMA RESEARCH

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ABSTRACT

In the development of new antiallergic or antiasthmatic therapies, mouse models have helped to identify novel therapeutic agents. Before a medication is evaluated for potency in phase II and phase III studies in humans, different bronchial challenge models are used to test the efficacy and mode of action in small sample sizes. Most published trials follow a classical approach in which allergic subjects are challenged with the same amount of allergen before and after treatment with a specific agent. Repeated challenge models are designed either to imitate natural allergen exposure or to induce significant asthma symptoms and airway inflammation. Although the available literature is less abundant, repeated models promise insights into the action of agents and the mechanisms of airway inflammation.

Keywords: Allergic asthma, bronchial allergen challenge, low-dose challenges, high-dose challenges.

INTRODUCTION

Specific bronchial allergen provocation is an established tool in asthma research that can increase our understanding of the pathological mechanisms responsible for allergic asthma and can offer key information concerning the therapeutic potential of new agents. Asthma research allows for the evaluation of antiallergic and antiasthmatic agents in relatively small sample sizes.¹⁻³ Subjects are typically challenged with an allergen before and after treatment with antiallergic or antiasthmatic drugs, and they are selected to develop reproducible early and late asthmatic responses (EAR and LAR, respectively). Another provocation model imitates natural allergen exposure. In the low-dose provocation model, a small amount of an allergen is inhaled to induce bronchial inflammation on consecutive days.⁴⁻⁶ Recently, studies have described high-dose challenge protocols^{3,7} as an interesting alternative to the existing protocols.

ANIMAL MODELS

Animal models have tremendously advanced the understanding of allergic disease development and

have helped to identify novel therapeutic agents.⁸ The mouse is now the species of choice,⁹ and the BALB/c strain exhibits a genetically determined tendency to develop Th2-biased immune responses.¹⁰ In mouse models, allergic asthma is typically induced by intraperitoneal injection of an antigen. After the sensitisation period, the mice are challenged with aerosol inhalation or nasal application of the antigen.⁸ In recent years, several groups have developed mouse models that can reproduce many of the features of the remodelled asthmatic airway.¹¹ Blyth et al.¹² noted a reduction in subepithelial reticulin and an almost complete depletion of airway eosinophilia, when given an anti-IL-5 antibody before allergen challenges. During chronic repetitive allergen challenges, IL-5 gene deletion suppresses lung eosinophilia and tissue remodelling, simultaneously.¹³ These animal studies strongly support the hypothesis that eosinophils contribute to the airway remodelling.¹¹ In mice, intranasal-challenged with house dust mite, both prophylactic and therapeutic treatment with an anti-IL-13 mAb significantly inhibited the generation and maintenance of chronic airway cellular inflammation, peribronchial collagen deposition, and epithelial goblet cell up-regulation.¹⁴

In comparison with human models, the mouse model has several disadvantages. Mice do not display spontaneous symptoms consistent with asthma.¹⁵ In mice, bronchial hyperresponsiveness (BHR) is only transient and does not, as in asthmatic subjects, appear during clinical remission since mouse airways do not contain smooth muscle bundles.^{11,15} The majority of studies have been performed with ovalbumin, but ovalbumin is not a clinically relevant allergen in humans.⁸ Challenges in mice predispose to nasal and alveolar response rather than directed to the lower conducting airways. In humans, the EAR alerts the individual to natural exposure to inhaled allergens.¹⁶ In contrast, in the LAR, eosinophils are important, and in response to IgE binding to the Fc[epsilon]RI receptor, eosinophil cytoplasmic granules and a number of cytokines are synthesised and released by degranulation.¹⁷ In mice, it is only in some models that allergen challenges involve either EAR or LAR.¹⁸ Allergen-specific IgE significantly predicts the LAR and EAR in mite-allergic asthmatic children and adolescents.¹⁹ However, in mice, IgE and mast cells are unnecessary for the generation of allergic asthma.²⁰ Moreover, allergen-driven murine models disregard other environmental factors of asthma, such as oxidant stress, viral infection, obesity, exposure to tobacco smoke, and pollutants.¹⁵

CLASSICAL CHALLENGE MODELS

The most common method of testing pharmaceutical agents is to challenge patients with the same dose of allergen after receiving treatment with the test drug and again after receiving treatment with placebo.¹⁶ Alternatively, randomised, placebo-controlled, parallel designs have been used²¹ in which allergen challenge is performed according to a standardised protocol. The allergen concentration that causes a 20% drop in FEV₁ (PC₂₀ allergen) is predicted from the PC₂₀ methacholine and skin test sensitivity, which is derived from a multi-dose skin prick test (SPT). Starting three concentrations below the predicted PC₂₀ allergen, consecutive doubling concentrations of allergen are aerosolised for 2 minutes using a DeVilbiss 646 nebuliser.²² The endpoint measurements in such studies are the maximal early and late percentage decreases in the FEV₁ and the areas under the curve in the EAR (0–2 hours post-challenge) and the LAR (3–7 hours post-challenge).¹⁶ The practical use of the protocols has been shown in numerous clinical trials.^{22–25} Allergen challenge studies can be of value to predict

efficacy or lack of efficacy of asthma controller therapies because agents that inhibit the LAR and allergen-induced inflammation are generally effective in asthma therapy.¹⁶ In the classical method, concerns exist because the predictive value of the SPT is limited. In adults allergic to cats, a positive SPT (wheal size 3 mm) failed to discriminate between challenge-positive and challenge-negative patients.²⁶ In house dust mite allergies, the skin sensitivity did not significantly contribute to the prediction of an EAR.²⁷ Consecutive doubling concentrations of allergen might not allow for the exact and equivalent timing of allergen administration between subjects, particularly in trials in which repeated allergen challenges are necessary to study the kinetics of anti-allergic drugs.²

LOW-DOSE CHALLENGE MODELS

Low-dose allergen challenges are designed to induce airway inflammation. Useful markers of inflammation are the induction of eosinophils and the eosinophilic cationic protein (ECP) in sputum and exhaled nitric oxide (eNO).^{4–6} In an initial incremental challenge with doubling doses or concentrations of an allergen, the dose/concentration is increased until the FEV₁ has fallen by 20% or more from baseline (PD/PC₂₀FEV₁). The dose that causes a 5% fall in the FEV₁ is determined during the screening allergen challenge. This dose is administered as a single challenge on 5 consecutive days.⁴ Trials with similar procedures have reported different outcomes, and not all trials have reported the presence of asthma inflammation caused by ‘silent’ chronic allergen exposure (Table 1). In repeated low-dose allergen provocations, night-time asthma symptoms and night-time β₂-agonist use significantly increased during the challenge period, and the PC₂₀ methacholine levels were significantly reduced.⁴ In a placebo-controlled study with inhaled steroids, 26 patients with mild asthma and mite allergy performed repeated inhalations of the PD₅ allergen for 2 consecutive weeks. Due to increased β₂-agonist use at day 2, the use was significantly elevated in the placebo group, whereas there were no significant differences in the total daily symptom scores. In the placebo group, the PC₂₀ methacholine levels did not decrease significantly after 2 weeks of allergen exposure.⁵ In a similar protocol, our working group showed that the participants did not require any β₂-agonists during the challenge period with house dust mite allergen despite the induction of allergic airway inflammation. The PD₂₀ methacholine levels decreased in

Table 1. Low dose challenges and symptoms.

Clinical trial	Author	Subjects (n)	Inhaled allergen	Duration of challenge	Allergen-dose	Cough	β2-agonist	BHR
Effect of n-3 polyunsaturated fatty acids in asthma after low-dose allergen challenge	Schubert et al., 2009	23	Mite	10 days	PD ₅	↔	↔	↔
Comparison of the effects of repetitive low-dose and single-dose antigen challenge on airway inflammation	Liu et al., 2003	8	Mugwort, Mite, Cat	4 days	PD ₅	NA	NA	NA
Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids	de Kluijver et al., 2002	26	Mite	10 days	PD ₅	↔	↑	↔
Airway inflammation and altered alveolar macrophage phenotype pattern after repeated low-dose allergen exposure of atopic asthmatic subjects	Lensmar et al., 1999	8	Birch, Grass	7 days	PD ₁₀	↔	NA	↑
Repeated aerosol exposure to small doses of allergen. A model for chronic allergic asthma	Arshad et al., 1998	9	Mite	12 days	0.4 ng	↑	↑	↑

BHR: bronchial hyperresponsiveness.

the placebo group, but the difference failed to reach significance.⁶ All reported studies⁴⁻⁶ have shown significant changes in sputum eosinophils and ECP during allergen challenges, and the eNO has been reported to increase stepwise to a peak level.⁶ Therefore, the low-dose allergen challenge is a perfect model for testing antiasthmatic or antiallergic agents in humans, and it has been used for budesonide⁵ and n-3 polyunsaturated fatty acids.⁶ In clinical asthma studies, the reduction of symptoms is one of the primary outcomes. The main point of criticism is that low-dose allergen challenges do not cause asthma symptoms.

HIGH-DOSE CHALLENGE MODELS

In a real-life setting, individuals may be exposed repeatedly to symptomatic doses of allergen,⁷ for

example cladosporium allergy,³ and so, in mouse models, acute sensitisation protocols include multiple systemic administrations of the allergen.¹⁸ To support previous findings, these mouse models should be validated against human responses.⁷ In the development of high-dose challenge protocols, Grainge and Howarth⁷ and our group³ independently designed very similar protocols. The Aerosol Provocation System (APS) dosimeter technique (Cardinal Health, Hoechberg, Germany) allows the computer-controlled production of an aerosol using a jet-type nebuliser to define an individual dose. The integrated pressure calibration procedure, associated with the compressor, ensures a highly constant and reproducible nebuliser output. In the incremental provocation, rather than doubling concentrations, a single allergen dilution with predefined doses is used. Both working groups chose the PD₁₅ allergen to

challenge mild asthmatics who were allergic to house dust mites. Whereas Grainge and Howarth⁷ used a more attenuated protocol with three consecutive challenges at 48 hour intervals, we hypothesised that four consecutive challenges in 1 week may be more likely to induce symptoms and allergen-driven asthma exacerbation in diseased volunteers. In both protocols, subjects developed significant asthmatic symptoms and rescue medication use. In the attenuated protocol, the pre and post-FEV₁ did not differ significantly and there were no serious adverse events, such as significant worsening of asthma requiring oral corticosteroids or hospital admission. In contrast, in our study the overall FEV₁ dropped significantly, and seven subjects had to stop the protocol prematurely; five patients experienced decreases in FEV₁ that were greater than that defined in the study protocol, and two had asthma attacks and required prednisolone during the night.

One of the primary outcomes of our study was sputum induction and the measurement of sputum cell counts and cytokines. We observed significant increases in the total eosinophil count, percentage of eosinophils, levels of ECP, and IL-5, which is a key mediator of eosinophil activation. In addition, transcription factor Foxp3 was significantly increased. In parallel, bronchial hyperresponsiveness, measured by methacholine challenge, and eNO demonstrated highly significant changes.³

In a bronchoalveolar lavage (BAL) study, the numbers of CD69+ and Foxp3+ lymphocytes were higher in the BAL fluid post-allergen provocation in asthmatic patients compared to pre-allergen provocation. To the best of our knowledge, we are the first group to demonstrate that Foxp3 is expressed in sputum cells after bronchial allergen challenge. The appearance of Foxp3 suggests the involvement of CD25+CD4+

Treg cells and a modulating role of Treg cells after allergen exposure, as Foxp3 CD4+CD25+Treg cells contribute to the control of allergen-specific immune responses in several major ways (e.g. the regulation of effector Th-1 and Th-2 cells).²⁸

High-dose challenge models are suitable to induce significant asthma symptoms in diseased volunteers. Sputum cell counts and cytokine levels are promising parameters for understanding new mechanisms in asthma and allergy regulation, and they are more precise than the measurement of bronchial hyperresponsiveness or eNO.^{29,30}

It is possible that the protocol we used was too intense because subjects developed severe asthma symptoms and decreases in pulmonary function. However, three high-dose challenges at 48 hour intervals are safe and repeatable.⁷ The high-dose challenge is a model for proof-of-concept studies in clinical settings to reduce the risk of severe asthma exacerbations.³

CONCLUSIONS

Different provocation models may answer different questions regarding the anti-allergic or anti-asthmatic action of new agents. Classical bronchial allergen challenges sufficiently demonstrate the efficacy of asthma controller therapies. Repeated low-dose allergen challenges cause airway inflammation and they are suitable for demonstrating the effects of medications in everyday life. As demonstrated in mouse models, high-dose challenge models validate the findings of basic research by both demonstrating the efficacy of a new agent and its anti-asthmatic potency and by investigating its impact on airway inflammation, as represented by sputum cell counts and sputum cytokine levels.

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