

## EDITORIAL

# Clinical & Experimental Allergy

## Allergen bronchoprovocation and chitinases in allergic asthma

This editorial discusses the findings of the paper in this issue by M. L. Gavala et al. [10], pp 187–197.

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Allergen bronchoprovocation is one of the few interventions which can induce a controlled exacerbation of disease in a laboratory setting, and it has been invaluable as a research tool for investigation of upper and lower respiratory tract allergic diseases. Bronchial challenges can be administered by inhalation of an allergen extract as a high dose challenge on a single day, as low doses of allergen across several days, or as a segmental challenge where allergen is instilled directly in the airways by bronchoscopy. All of these challenges are well standardized and induce clinical expressions of asthma, including variable airway obstruction and airway hyperresponsiveness, which are associated with airway inflammation and structural alterations in the airways.

Several key components of the allergen-induced inflammatory cascade and their contribution to pathology in asthmatic airways are well understood. Initiation of acute allergen-induced bronchoconstriction requires sensitization to antigen which results in circulating IgE to inhalant antigens such as *Der p1* (house dust mite), *Fel d1* (cat dander) and/or many others. Inhalation of these and other antigens leads to cross-linking of the allergenic epitopes with allergen-specific IgE which is bound to airway mast cell and basophil high-affinity IgE receptors. The subsequent activation of secretory pathways causes release of both pre-formed and newly generated mediators of bronchoconstriction and vascular permeability into the airways. These mediators include histamine, lipid mediators and a wide array of cytokines and chemokines. A late-phase response can also develop follow-

ing allergen bronchoprovocation, resulting from a complex interaction of antigen-presenting cells and T lymphocytes which drives Th2-mediated cellular inflammation and release of cytokines, such as IL-4, IL-5 and IL-13. This Th2 cellular inflammation recruits effector cells such as eosinophils, mast cells, basophils and Th2 lymphocytes to the airways. Activation of these leucocytes leads to bronchoconstriction and airway hyperresponsiveness resulting from increased bronchovascular permeability, mucus secretion and bronchial remodelling [1–3]. The bronchial remodelling associated with allergen bronchoprovocation corresponds to an increased thickening of the bronchial wall caused by various structural alterations including abnormal epithelium, subepithelial membrane thickening, alteration in extracellular matrix deposition, mucus gland hypertrophy and increased mass of bronchial smooth muscle [4].

Allergen bronchoprovocation has been a fundamental research tool used for the identification of mediators of inflammation and their cellular sources in allergic asthma. Allergen-induced airway inflammation was first documented by deMonchy in 1985 using bronchoscopic lavage [5]. Airway inflammation can also be measured less invasively by examining inflammatory cells from samples of induced sputum [6]. Whether measured in sputum, bronchioalveolar lavage (BAL) or airway tissue, eosinophilia is a consistent finding following allergen bronchoprovocation. Mechanisms of allergen-induced cellular recruitment have been teased apart through the identification of mediators, which increase following bronchoprovocation with allergen but not following bronchoprovocation with diluent control. Findings of increased levels of mediators such as IL-5 and IL-13, together with their pivotal role in animal models of allergic disease, have led to novel targets for asthma therapies such as monoclonal antibodies mepolizumab and lebrikizumab which are currently in clinical development.

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Inhaled allergen challenge and segmental challenges have also been used to test new asthma therapies, providing key information on the therapeutic potential of new agents and on their anti-inflammatory effects on the airways. An assessment of a drug's ability to block allergen-induced responses provides essential evidence showing activity in the airways. Effects of a therapy on airway inflammation and remodelling can be examined using either the segmental or inhaled allergen models, while the effects on the early and late-phase asthmatic responses and airway hyperresponsiveness can only be assessed with the inhaled allergen challenge model; subjects are challenged with exactly the same dose of allergen after receiving treatment with the test drug, and again after receiving treatment with placebo. Reproducibility of measurements shows that no more than eight subjects are usually required in a cross-over study design to show a 50% attenuation of the early asthmatic response, late asthmatic response or airway eosinophilia, with a 90% statistical power [7, 8].

The many components of the allergic cascade and the mechanisms by which allergens contribute to inflammation and remodelling in the airways continue to be revealed using allergen bronchoprovocation models. Chitin, a polysaccharide commonly found in lower organisms, has been shown to have effects on both the innate and adaptive immune responses, including the ability to mobilize and activate innate immune cells, and to induce cytokine and chemokine production [9]. Gavala et al. measured chitinases in the airways of subjects with mild allergic asthma, after undergoing segmental allergen bronchoprovocation [10]. Specifically, they measured the activity of chitotriosidase, the enzyme which digests chitin, and the level of YKL-40, a chitinase-like protein which lacks chitinase activity due to mutations within the active site. Chitotriosidase is secreted by activated human macrophages and may play a role in the degradation of chitin-containing pathogens. Its enzymatic activity is markedly elevated in serum of patients suffering from lysosomal lipid storage disorders [11]. The physiological role of YKL-40 is not known.

Chitinases have been linked to Th2-mediated inflammation, [12] and elevated levels of YKL-40 have been noted in a variety of human diseases including asthma, where they correlate with disease severity and airway remodelling [13]. Chronic structural changes in the airway of subjects with asthma include goblet cell metaplasia, deposition of extracellular matrix in the

submucosa, smooth muscle hyperplasia/hypertrophy and hyperplasia of fibroblasts/myofibroblasts [14]. It is believed that airway remodelling plays an important role in the pathophysiology of airway hyperresponsiveness, and may be related to the development of fixed airflow obstruction in individuals with long-standing chronic asthma [4]. It was previously established that YKL-40 is secreted from epithelial cells and macrophages of patients with severe asthma, and the gene encoding YKL-40 has been associated with airway hyperresponsiveness and impaired lung function, [15] also suggesting involvement with remodelling of bronchial smooth muscle.

Most of the literature involving chitin, chitinases and asthma are based on murine models, thus it is essential to determine whether these findings are also applicable for humans. Gavala et al. demonstrated that BAL cells express chitotriosidase and YKL-40. They also demonstrated that the activity of chitotriosidase in BAL fluid increased approximately 10-fold at 48 hours after segmental allergen bronchoprovocation, and the levels of YKL-40 levels measured in BAL fluid were also significantly elevated post-challenge. Furthermore, measurements of these chitinases at 48 hours post-allergen correlated positively with other pro-fibrotic factors, including fibronectin, insulin-like growth factor binding protein-3 and MMP-9. Interestingly, thrombin correlated with chitotriosidase activity and YKL-40 levels both at baseline pre-challenge, and at 48 hours post-challenge [10]. These data are the first to show that chitotriosidase activity is increased in human lungs after *in vivo* allergen exposure and support the notion that allergen challenge leads to increased chitotriosidase activity which correlates with profibrotic factors in the lungs. These findings strongly support the concept that chitinases contribute to airway remodelling in asthma.

As with all allergen bronchoprovocation studies, however, there are some limitations to the interpretation of data generated. Subjects that are suitable for these studies have mild allergic asthma, so it is not known whether these findings can be extended to those with more severe disease, or applicable to non-allergic asthma. However, this new evidence strongly supports previous reports showing a connection between chitinases and airway remodelling in allergic asthma, and this relationship warrants further investigation.

*Conflict of interests:* The author declares no conflict of interests.

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