Allergen bronchoprovocation test

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Allergen bronchoprovocation test: an important research tool supporting precision medicine

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Purpose of review
Allergen bronchoprovocation test (ABT) has been used to study asthma pathophysiology and as a disease-modelling tool to assess the properties and efficacy of new asthma drugs. In view of the complexity and heterogeneity of asthma, which has driven the definition of several phenotypes and endotypes, we aim to discuss the role of ABT in the era of precision medicine and provide guidance for clinicians how to interpret and use available data to understand the implications for the benefits of asthma treatment.

Recent findings
In this review, we summarize background knowledge and applications of ABT and provide an update with recent publications on this topic. In the past years, several studies have been published on ABT in combination with non-invasive and invasive airway samplings and innovative detection techniques allowing to study several inflammatory mechanisms linked to Th2-pathway and allergen-induced pathophysiology throughout the airways.

Summary
ABT is a valuable research tool, which has strongly contributed to precision medicine by helping to define allergen-triggered key inflammatory pathways and airway pathophysiology, and thus helped to shape our understanding of allergen-driven asthma phenotypes and endotypes. In addition, ABT has been instrumental to assess the interactions and effects of new-targeted asthma treatments along these pathways.

Keywords
allergen bronchoprovocation test, asthma, inflammation, precision medicine

INTRODUCTION

Asthma is a complex and heterogeneous disease presenting as various phenotypes or endotypes [1,2]. The recognition of such variety of clinical presentations and underlying mechanisms has led to the concept of precision medicine, often described as providing the right treatment to the right patient with asthma at the right dose without noteworthy side-effects and at the right time [3,4]. In the past decades, there have been significant advances in our understanding of asthma pathophysiology, which drove the development of new-targeted drugs for asthma. Given its unique disease-modelling characteristics in combination with an overall good predictive value [5], the allergen bronchoprovocation test (ABT), also named allergen inhalation test or allergen bronchial challenge, has substantially helped to shape our insights into asthma pathophysiology and potential targets resulting in new therapeutic approaches [6,7].

Linking the pathophysiology to the underlying inflammatory pathways and by enabling evaluation of the effects of interventions along the allergen-driven Th2-pathway, the ABT has thus substantially contributed to the development of precision or 'personalized' medicine in asthma and the development of targeted treatments [5,8]. Apart from the application of allergen exposure or challenge tests in

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KEY POINTS

- ABT is a useful tool to study asthma pathophysiology, inflammatory pathways and their relationships.
- ABT is a reproducible and repeatable research tool, and it should be performed only by qualified and experienced investigators with adequate expertise.
- Recent studies in allergen-sensitized patients with asthma helped to further define and refine our understanding of the allergen-induced type 2 inflammatory pathways and the effect of targeted treatments on several clinical, pathophysiological and inflammatory outcomes.
- New imaging methods and molecular biomarkers analysis including gene polymorphisms characterization helped to better understand underlying mechanisms and predict the pattern of asthmatic response.
- ABT have been used with success to assess the therapeutic potential of various new targeted molecules and their mode of action in patients with specific phenotypes and endotypes, and hence, ABT can contribute to precision medicine in specific populations.

the diagnosis of occupational asthma, the ABT is mainly considered as a research tool [9]. In this review, we summarize what is known about these tests and provide an update with recent publications on this topic and discuss how the clinician can interpret and use these data to understand the implications for the benefits of asthma management/treatment in the era of precision (or personalized) medicine. We did not include animal studies, as their relevance to the human situation is often uncertain, although they may be useful to generate hypotheses and explore specific mechanisms.

A standardized method for experienced researchers

ABT is a standardized and validated procedure that induces airway changes consistent with symptoms and features of asthma. The test outcomes are repeatable and reproducible within patients, which allows the assessment of underlying asthma mechanisms and drug effects in small sample sizes (8–14 patients) [5]. However, because this methodology causes a transient asthmatic reaction and as its magnitude is subject-specific, the test requires ample expertise and qualified professionals to ensure its safety and valid outcomes.

In allergen-sensitized patients with asthma, ABT usually induces an acute bronchoconstriction within 10–20 min following the provocative allergen dose [early asthmatic response (EAR)], which is a transient, mainly a bronchoconstriction phase. In approximately 50% of patients, the EAR is followed by another phase of bronchoconstriction, the late asthmatic response (LAR), usually occurring between 3 and 8 h after the challenge [10]. The LAR is associated with a type 2 (or Th2) airway inflammatory response [11], and a variable increase in airway hyperresponsiveness (AHR) [12].

There are different types of allergen bronchoprovocation tests of the lower airways: that is, the 'whole lung' or 'total lung' allergen challenge being most often used, following inhalation of incremental doses of allergen extract in a laboratory setting, although 'real-life' allergen exposure chambers (AEC) are also being used in specialized research facilities [13]. AECs allow simultaneous controlled exposure of multiple subjects to allergens in a closed environment [13,14] but is not ideally suited for whole lung challenges as the provocative allergen dose is subject-specific. Both the whole lung challenges and AEC require careful titration of allergen to avoid overdose leading to a severe bronchoconstriction. Segmental allergen challenge (SAC), is another, albeit less frequently used bronchoprovocation method, which requires the invasive procedure of bronchoscopy, although it is considered safe in experienced hands [15]. SAC involves higher doses of allergen instilled into an isolated segment of the lung, and thus allows a more aggressive and invasive assessment of the allergen-induced airway inflammation including bronchoalveolar lavage, bronchial brushes and/or bronchial biopsies. Limitations of SAC include the invasive nature of the procedure leading to a restriction in the number of bronchoscopies/sample collections that can be performed during the testing period, and the inability to measure changes in airway physiology because of the effect of confounding medications administered during the bronchoscopy procedure and the procedure itself.

A useful model to investigate asthma pathophysiology and underlying pathways

In the last decades, allergen challenges have been used to assess various mechanisms involving allergic airway responses, both at the level of large-medium and small airways [16,17]. Especially when combined with (non or semiinvasive) airway samplings, the ABT has substantially contributed to our understanding of the pathophysiology and underlying pathways of asthma and consequently helped to define its phenotypes and endotypes [18]. As such, the model has also proved to be suitable for the evaluation of various (targeted) asthma therapies [19].

ASTHMA CLINICAL FEATURES AND PATHOPHYSIOLOGY

Effect of allergen bronchoprovocation test on cough reflex

Satia *et al* [20]. studied the effects of allergen exposure on cough reflex sensitivity. In a nine-visit, randomized, single-blind, diluent-controlled, two-way crossover study, cough responses to inhaled capsaicin were analyzed in 12 patients with corticosteroid-naïve, mild allergic asthma following whole lung inhaled allergen challenge [20]. In addition to the development of allergen-induced bronchoconstriction and airway eosinophilia, there was an increase in capsaicin-evoked coughs after allergen inhalation compared with diluent both at 30 min and 24-h postallergen challenge and an increase in spontaneous coughs over the 24-h postallergen challenge. This suggests that the allergen-induced airway obstruction/hyperresponsiveness and airway inflammation contribute to heightened cough reflex sensitivity.

Airway inflammation following allergen bronchoprovocation test

Airway inflammation can be explored directly using invasive techniques such as bronchoscopy, but non-invasive sputum induction and analysis allow more frequent assessments. Indirect surrogate markers, such as exhaled breath analysis including fractional exhaled nitric oxide, also allow more airway samplings that are easier to collect but more difficult to interpret [21]. Direct assessments of airway inflammation from sputum samples have been shown to reflect the severity of the asthmatic response (e.g., isolated early asthmatic responses versus a prolonged and/or dual asthmatic responses, this last usually showing more intense airway inflammation) [22,23] and to assess the response to antiinflammatory [24] or proinflammatory therapy [25]. Sputum sampling is a method that provides highly reproducible measurements of allergen-induced inflammatory cell differentials, especially eosinophils and neutrophils [12]. Sputum induction can be conducted repeatedly after whole lung challenge to examine the kinetics of various inflammatory cells [26]. Using microscopy, levels of mast cells and basophils in sputum have been shown to peak within 7 h after allergen challenge [27] whereas eosinophils peak between 7 and 24 h after allergen challenge and resolve over a period of days [26]. Rare cell types including dendritic cells, T-cell subsets, Tregs, B-cell subsets, progenitor cells and ILC2 cells [28–34] can be quantified by flow cytometry to identify new inflammatory axes [35–37] and demonstrate an inflammatory cell time course consistent with cytokine and chemokine accumulation in the airways postchallenge [38].

Although not fully clarified, club cell protein (CC16), a pneumoprotein produced by nonciliated club cells within the airway epithelium, is thought to exert antiinflammatory and immunomodulator effects within the lungs. Stenberg *et al.* [39] investigated the effect of inhaled ABT on levels of pulmonary and systemic CC16 in 34 patients with allergic asthma. Compared with preallergen, CC16 levels in plasma increased in all patients within one hour postchallenge, whereas in the bronchoalveolar lavage fluid CC16 was increased only in the mono-responders (no LAR) at 24 h postallergen. These findings suggest that plasma CC16 levels following (inhaled) proinflammatory stimuli are a potential biomarker of airway epithelial damage/dysfunction and that a lack of increase in pulmonary CC16 postallergen may explain the reduced antiinflammatory (or protective) response in patients with a dual response to inhaled allergen.

Furthermore, Kalinauskaite-Zukauske *et al* [40]. investigated serum levels of IL-25, IL-33, thymic stromal lymphopoietin (TSLP), ezrin, IL-4 and IL-13, following bronchial challenge with *Dermatophagoides pteronyssinus* in patients with allergic asthma. In corticosteroid-free, clinically stable, asthmatic patients sensitized to *D. pteronyssinus* (allergic asthma) compared with nonatopic healthy patients [40]. Baseline levels of IL-25, TSLP and ezrin did not differ between the allergic asthma and healthy patients groups. Following allergen exposure, significant increases in serum IL-25, TSLP and ezrin levels were observed in patients with allergic asthma. Although at baseline serum levels of IL-33 were significantly higher in the allergic asthma group compared with healthy patients, allergen challenge did not cause any further increase in this cytokine in any group. Serum IL-4 and IL-13 levels were significantly higher at baseline in the allergic asthma group compared with healthy patients, with further increases postallergen in the allergic asthma group, but not in healthy patients. It was therefore concluded that the epithelial-derived mediators IL-25, TSLP and ezrin, enhance type 2 inflammation via IL4/IL13 signalling after bronchial challenge with *D. pteronyssinus* in allergic asthma. This is consistent with previous findings [11]. Measurements in endobronchial biopsies 24 h after whole lung allergen challenge also show increases in IL-25, IL-33, ST2 and TSLP [41,42]. Supportive studies demonstrate enhanced expression of IL-25 and IL-25 receptor in mature and immature eosinophils [43] and after whole lung allergen challenge, there is increased plasma and sputum IL-33 levels as well as significant

upregulation of ST2 surface expression on eosinophils from blood and sputum [44].

Sputum RNA signature after allergen exposure: effects of inhaled corticosteroids

Zuiker *et al.* [11,18] studied the effect of inhaled allergen challenge with house-dust mite (HDM) extract on the RNA signature from induced sputum [18] and the kinetics of Th2 biomarkers using innovative detection techniques [11]. Additionally, the effect of pretreatment with fluticasone 500 mcg BID (a total of five doses) on the allergen-induced changes in lung function, sputum RNA signature and airway inflammation (sputum inflammatory cells and Th2-derived cytokines and chemokines) was assessed. This randomized, placebo-controlled, two-period crossover study was performed in 13 nonsmoking, allergic patients with clinically stable, corticosteroid-free, mild-to-moderate asthma [11,18] with a demonstrated LAR to inhaled HDM extract. Inhaled HDM extract induced a LAR in all patients and upregulated the expression of several genes along the Th2-pathway consistent with increases in sputum Th2-cytokines (IL4, IL5, IL13 and eotaxin-1) and eosinophils both at 7 and 24 h postallergen. These allergen-induced changes in airway physiology and inflammation as well as the gene-expression were blunted by fluticasone. These data show that these new techniques may allow to study inflammatory signatures in noninvasive (semiinvasive) airway samplings such as induced sputum and quantify drug effects on this response in allergic asthmatic patients.

The team led by Tebbutt looked at genes and gene polymorphisms in isolated early responders versus dual responders to whole lung allergen challenge [45,46,47▪▪,48]. They identified 64 uniquely expressed transcripts in whole blood that reflected a variety of innate, humoral and adaptive immune processes, and 13 uniquely expressed transcripts in peripheral blood mononuclear cells (PBMCs), which were representative of T-cell and monocyte-mediated processes. In mild allergic asthmatics compared with nonasthmatics, 47 differentially expressed transcripts were identified in whole blood compared with one differentially expressed transcript in PBMCs (False Discovery Rate < 0.25). Furthermore, the Trinity biomarker panel was useful in predicting the response of patients that elicited different responses and patients that elicit a dual response upon repeated allergen inhalation challenges, suggesting that these biomarker-blood tests may be used to identify patients with asthma who develop the LAR. These tests may reveal novel molecular mechanisms that can be targeted for therapy. In another study, these authors suggested that the variability in the onset of the late asthmatic response might explain the poor predictive performance of the postchallenge multiomic biomarker signature. Further work should shed light on the molecular mechanisms involved and the utility of the above tests [45,46,47▪▪,48].

Innate lymphoid cell response following allergen responses

Innate lymphoid cells (ILC2s) produce IL5 and IL13 during allergic inflammation and are considered to bridge innate and adaptive immune responses [49,50]. ILC2 numbers are increased in asthmatic patients compared with healthy control patients [51] and higher in patients with severe eosinophilic asthma compared with mild allergic asthma [52]. Winkler *et al.* [53▪] compared blood and lung-derived ILC2s before and after a SAC in patients with mild-to-moderate asthma with high blood eosinophil counts ($\geq$300 cells/ml). ILC2s were almost absent in the alveolar space under baseline conditions, but their numbers increased significantly 24 h after allergen challenge, whereas at the same time, their numbers decreased in the blood. Prostaglandin D_2 and CXCL12 levels in BAL fluid correlated with the decrease in ILC2 cells. After allergen challenge, several genes promoting type 2 inflammation were expressed at greater levels in BAL fluid compared with blood ILC2s, whereas blood ILC2s remained inactivated. The authors concluded that ILC2s are recruited from the blood and can accumulate at the site of the allergic inflammation, their transcriptional and functional activation pattern promoting type 2 inflammation. Furthermore, Chen *et al.* observed increases in IL5+, IL13+ and CRTH2+ ILC2 cells in sputum at 24 h after whole lung allergen challenge; sputum ILC2 levels correlated with sputum airway eosinophilia and were coincident with a decrease in circulating ILC2, suggesting that airway-specific increases in ILC2 drive airway eosinophilia.

The role of neutrophils in allergen challenge revisited

Although the role of neutrophils in allergic airway disease has been ignored for a long time and is currently still not fully understood, recent evidence points toward involvement of specific neutrophil subsets in the inflammatory events induced by allergens [54]. Ekstedt *et al.* [55] studied neutrophil subsets (CD16 and CD62L) in peripheral blood 24 h after ABT in nine allergic asthmatic patients who participated in a large allergen challenge exploratory study. Five of them were mono-responders,

whereas four had a dual airway response following inhaled allergen. Seven of them could be classified as eosinophilic asthma phenotype (blood eosinophils > 150 cells/mcl). At baseline, all of the patients had less than 40% activated blood neutrophils. At 24 h postallergen, there was a shift from nonactivated mature neutrophil subsets: CD16 (high) CD62L (high) to activated mature neutrophil subsets CD16 (high) CD62L (dim), which previously have been shown to play a role in airway hyperresponsiveness [55]. A retrospective analysis of more than one hundred patients undergoing whole lung allergen challenge also demonstrated that the number of both eosinophils and neutrophils in sputum increased significantly at 7 h and remained elevated at 24 h postchallenge [38]. Interestingly, those with paucigranulocytic sputum were found to readily develop eosinophilic or mixed granulocytic sputum after allergen challenge, suggesting that this asthma phenotype is dynamic rather than static.

Allergen bronchoprovocation test and airway remodelling

Allergen-induced late responses are known to induce airway hyperresponsiveness [56] and airway remodelling [57]. Recently, Adams *et al*. [58] studied 20 allergic individuals with mild asthma, 22 nonasthmatic allergic controls and three healthy controls. Before and 24 h following a segmental allergen challenge to the right middle lobe, a 3-cm airway segment in the right middle and right upper lobe was imaged with endobronchial optical coherence tomography. Epithelial thickness and mucosal buckling had a significant relationship to forced expiratory volume in 1 s over forced vital capacity (FEV1/FVC) in the asthmatic group, suggesting that a microstructural approach to assessing the airways may eventually be useful to investigate physiopathological changes following stimuli or the effects of specific interventions (e.g., with medications) on airways structure without exposure to radiation.

In regard to imaging the airways after allergen challenge, we previously reported increased airway wall thickness assessed at the intermediary bronchus level on chest tomodensitometry following inhaled allergen challenge and suggested that this was secondary to the acute allergen-induced airway changes such as airway wall oedema, observed during the late asthmatic response [59].

Allergen bronchoprovocation test and small airways

As mentioned before, apart from (airway) inflammatory changes, ABT has been shown to induce airway remodelling (57), which also includes small airways. However, the allergen-induced airway responses have been traditionally defined in terms of FEV1, which is insensitive to changes in small airways pathophysiology. Therefore, the relationship between several aspects of the large and small airways function following ABT comparing mono-responders ($n = 19$) and dual responders ($n = 15$) was assessed in allergic asthmatic patients [16]. Airway response was evaluated with spirometry, impulse oscillometry (IOS), body plethysmography, inert gas washout and single breath methane dilution carbon monoxide diffusion at several timepoints from preallergen up to 23 h postallergen challenge. Peripheral airway resistance, air trapping and ventilation heterogeneity were significantly increased in dual responders compared with those with mono-response only, suggesting that dual responders have more extensive airway disease. The authors suggested to include small airways measures in future studies to link pathophysiology and inflammatory pathways from the different airways compartments and further refine the allergen challenge model. These findings are in line with previous observations that IOS is more sensitive than other lung function tests both after direct (methacholine) and indirect (allergen) bronchoprovocation tests [60].

ASSESSMENT OF POTENTIAL NEW ASTHMA THERAPIES

The development of novel anticytokines targeting a specific mechanism has provided a new avenue of research on how better target specific asthma populations. Whole lung allergen challenge was originally characterized using inhaled budesonide and found to reflect the antiinflammatory properties of corticosteroids through a reduction in late asthmatic response and sputum inflammatory cells [24]. With respect to testing of investigational therapies, allergen inhalation test was initially used to assess the first monoclonal antibody, omalizumab, directed at IgE, in the treatment of allergic asthma [61,62]. This study was followed by several others using inhaled allergen challenge as a proof of concept to evaluate small molecule inhibitors [63–69], novel corticosteroids, oligonucleotides and monoclonal antibodies [70–77].

More recently, the inhibition of allergen-induced asthmatic responses and secondary airway inflammation has been demonstrated for tezepelumab, an anti-TSLP [6,76] and for CSJ117, an anti-TSLP antibody fragment, thereby supporting a key role for TSLP in allergen-induced responses [78]. Effective asthma drugs have usually inhibited

allergen-induced responses and increases in sputum eosinophil counts associated with LAR inhibition.

Choosing the right population to test

Overall, the inhibition of the LAR and associated inflammatory changes and AHR have been used to effectively predict the efficacy of asthma-controlling agents. This was particularly the case for inhaled corticosteroids [11,18,24].

Initial studies on anti-IL5 such as mepolizumab showed the importance of testing drugs on a targeted population [66]. Indeed, the initial clinical studies in a general population of mild asthmatic patients showed that although it had some protective effects against allergen exposure, large scale studies without phenotyping had shown no significant benefit, whereas in patients with the specific underlying mechanism guided by airway eosinophilia, it could reduce asthma exacerbations by half [68,79–83]. Similar evidence was replicated in studies with CRTH2 antagonists' setipiprant and timapiprant [68]. Hence, ABT can help determine if a specific molecule can be effective in a population with the specific phenotype or endotype targeted by this drug.

Recent studies on new drugs

Revez *et al.* [70] tested the hypothesis that tocilizumab (TCZ), a human monoclonal antibody that blocks IL-6 signalling, can prevent the development of allergen-induced bronchoconstriction in humans in a randomized, double-blind, placebo-controlled study. Allergen inhalation tests were conducted before and after treatment with a single dose of TCZ or placebo. Patients were randomized to the TCZ ($n = 6$) or placebo ($n = 5$) groups. The primary efficacy endpoint, the magnitude of the late asthmatic response and the secondary efficacy endpoint, the early asthmatic response were not significantly different between the two groups. Therefore, there was no evidence that a single dose of tocilizumab was able to prevent allergen-induced bronchoconstriction. Furthermore, TCZ did not affect the sputum eosinophils or neutrophils.

FUTURE RESEARCH

We are now in an era of personalized medicine and last decades research has provided some insight about the future challenges about understanding allergic diseases and on how to optimize therapy [84▪▪]. In combination with innovative imaging, physiological and immunological techniques, ABT can help pursue this task and bring invaluable data on key questions.

CONCLUSION

ABT is a powerful research tool, which has not only been useful to study the pathophysiology of asthma and help to define its phenotypes and endotypes, but also to assess targeted therapies according to key-asthma related mechanisms. In the future, it should be part of the methods to assess new (targeted) molecules developed for asthma and respiratory allergy.

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This review provides an excellent overview of clinical applicability of point-of-care biomarkers to guide targeted therapeutic options in precision medicine and discusses future research targets and developments in asthma.