Inhaled allergen bronchoprovocation tests

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The allergen bronchoprovocation test is a long-standing exacerbation model of allergic asthma that can induce several clinical and pathophysiologic features of asthma in sensitized subjects. Standardized allergen challenge is primarily a research tool, and when properly conducted by qualified and experienced investigators, it is safe and highly reproducible. In combination with validated airway sampling and sensitive detection techniques, allergen challenge allows the study of several features of the physiology of mainly T_H2 cell–driven asthma in relation to the kinetics of the underlying airway pathology occurring during the allergen-induced late response. Furthermore, given the small within-subject variability in allergen-induced airway responses, allergen challenge offers an adequate disease model for the evaluation of new (targeted) controller therapies for asthma in a limited number of subjects. In proof-of-efficacy studies thus far, allergen challenge showed a fair positive predicted value and an excellent negative predictive value for the actual clinical efficacy of new antiasthma therapies, underscoring its important role in early drug development. In this review we provide recommendations on challenge methods, response measurements, sample size, safety, and harmonization for future applications. (J Allergy Clin Immunol 2013;132:1045-55.)

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Asthma and allergy are interrelated disorders with an increasing prevalence worldwide.[1,2] In sensitized asthmatic patients exposure to relevant allergens induces the development and persistence of airway inflammation.[3] These sequelae are known to be associated with airway hyperresponsiveness (AHR) to nonspecific triggers and loss of asthma control.[4]

For almost 3 decades, standardized allergen bronchoprovocation testing or allergen challenge has served as a validated model mimicking the acute and some of the more chronic features of asthma in human subjects,[5,6] which might differ in several aspects from animal allergen challenge models.[7,8] In research settings allergen challenge permits the study of various features of T_H2 cell–driven asthma and the evaluation of (targeted) asthma therapies.[9-12] Specific allergen challenge is routinely used in the investigation of occupational sensitization but otherwise is primarily a research tool that should only be conducted in specialized centers with demonstrable expertise and experience with this clinical model.

More recently, standardization and optimization of noninvasive airway sampling techniques and several biomarker detection methods have added to the interest in this specific asthma model.[13] This has driven the development of various challenge methods conducted in clinical research settings both inside and outside the hospital environment.[14,15] Unlike provocation tests with direct bronchoconstrictors, such as methacholine and histamine, which produce short-lived transient bronchospasm through direct interaction with receptors on airway smooth muscle, allergen challenge is an indirect test inducing prolonged bronchoconstriction through the release of proinflammatory mediators (Fig 1).[3] In sensitized subjects inhalation of a provocative dose of a relevant allergen is known to produce acute bronchoconstriction (ie, the early asthmatic response [EAR]), which in approximately 50% of cases is followed by an LAR. The LAR is characterized by an inflammatory airway response, prolonged airway narrowing, and AHR that can last for days to weeks (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org).[3,4,9] Therefore inhaled allergen challenge involves more complex procedures and requires more onsite expertise than just a well-written standard operating procedure (SOP) for safe and reproducible conduct.

Presently, there are 3 types of allergen challenges that can be applied within the respiratory tract: (1) nasal challenge, (2)

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Abbreviations used
AHR: Airway hyperresponsiveness
APC_{20}: Allergen concentration causing a decrease in FEV_1 of 20% from baseline
AUC: Area under the curve
EAR: Early asthmatic (allergic) response
eNO: Exhaled nitric oxide
LAR: Late asthmatic (allergic) response
SOP: Standard operating procedure

segmental lung challenge, and (3) total lung or inhaled challenge. The latter includes incremental and bolus (ie, high-dose) allergen challenges,[5,14] repeated low-dose allergen challenge,[15] and "real-life" allergen exposure rooms.[16] Despite similarities across the methodologies[17] and airway compartments,[2] the methods are not interchangeable because each challenge addresses different inflammatory pathways, pathophysiologic pathways, or both.

This review focuses on methodological aspects, safety, and the main applications of the inhaled allergen challenge. Because allergen challenge is increasingly applied in different research settings, it is important to optimize the safety of the methods used, as well as the comparability of data across study centers. Historical background on allergen bronchoprovocation is provided in the text in this article's Online Repository at www.jacionline.org.

SAFETY PRECAUTIONS

Although generally well-tolerated, occasionally, inhaled allergen challenge can induce severe (acute) bronchoconstriction or even generalized anaphylaxis, requiring urgent medical intervention. Therefore, and in agreement with good clinical practice, allergen bronchoprovocation tests should only be performed in centers with demonstrable expertise and ample experience with the required methodologies and subject populations.[18]

In any center undertaking inhaled allergen challenge, adequate knowledge of the pathophysiology and management of asthma should be in place. These training aspects should be obvious from updated curriculum vitae; training records, training certificates, or both; and/or publications. Apart from dedicated research staff, there should always be immediate access to an experienced (pulmonary) physician with demonstrable knowledge of asthma who is capable of managing acute bronchoconstriction and anaphylaxis. This physician should always be aware that a challenge is being conducted and be present during or in close vicinity to the actual challenge. All members of the team should be trained in resuscitation methods, and an updated crash cart should be close at hand, along with clearly written SOPs for anaphylaxis and acute bronchoconstriction management, including the emergency telephone number of the hospital. The contents of the crash cart should include cardiopulmonary resuscitation equipment, intravenous fluids (including a plasma expander and normal saline), adrenalin, antihistamines, and corticosteroids for parenteral use, along with sterile injection fluid (NaCl 0.9%), inhaled and intravenous bronchodilators (both short-acting β_2-agonists and anticholinergics), a nebulizing device, a ready-to-use oxygen source and mask, needles, and syringes.

The subject should never be left unattended during or after the challenge procedure, and FEV_1 should be closely monitored for at least 7 hours after challenge. After the last FEV_1 measurement (≥7 hours after challenge), subjects should receive inhaled bronchodilators until the FEV_1 returns to within approximately 10% of the preallergen baseline value. Once this is achieved and the subject is clinically stable, subject can be sent home and provided with the following:

- transportation from the research center to his or her home address, preferably without being left home alone;
- a bronchodilator (preferably a metered-dose inhaler in combination with an aerochamber) and an oral corticosteroid, including instructions on use;
- clear (both oral and written) instructions on the possibility of postchallenge recurrence of bronchoconstriction and its management with inhaled bronchodilator use; and
- emergency contact information of the on-call qualified physician who has been notified about the subject.

To prevent sensitization, bronchoconstriction, or both in susceptible investigators, an exhaust hood, high-efficiency particulate air (HEPA) filters, or both should be used during allergen nebulization, and the room should be adequately ventilated after the procedure.

Other good clinical practice–based prerequisites relating to safety and data quality and integrity are as follows:

- close vicinity to a hospital with an operational intensive care unit;
- adequate, well-ventilated challenge rooms with standardized humidity conditions within an irritant-free and (tobacco) smoke-free area;
- regularly calibrated and serviced equipment meeting American Thoracic Society/European Respiratory Society criteria;
- standardized validated SOPs;
- adequate databases; and
- a qualified laboratory and pharmacy complying with locally required standards.

In a multicenter setting ample attention should be paid to prior harmonization of the SOPs and equipment across the participating centers.

SUBJECT INCLUSION/EXCLUSION CRITERIA

Well-defined subject inclusion and exclusion criteria before allergen and continuation criteria on repeat challenges are required to ensure subjects' safety and data integrity.[18] Allergen challenge should never be performed in patients with severe asthma, unstable asthma, or both. Recommendations are summarized in Table I (also see Table E1 in this article's Online Repository at www.jacionline.org).

ALLERGEN INHALATION METHODS

Background

Allergen administration into the lung can be done by using several inhalation protocols, as described below. Although all inhaled challenge methods highlight some aspects of the allergic airway response, the "provocative inhaled dose" methods combine the advantage of studying both the allergen-induced inflammatory sequelae and the subsequent changes in asthma physiology.

Selection of a method and concentration for allergen inhalation is governed by 2 important concerns.

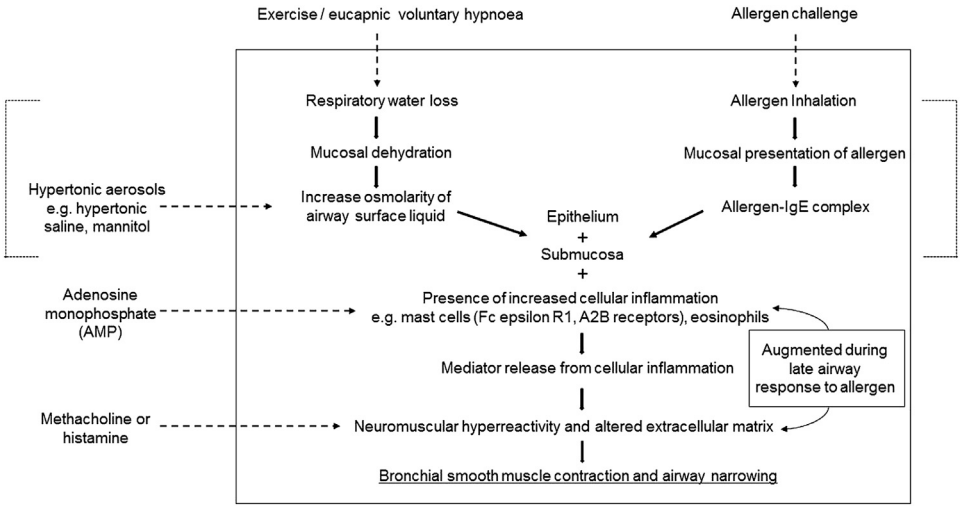

FIG 1. Mechanism of action of various standardized airway challenges applied in clinical practice and clinical research. Modified from O'Byrne et al.[3]

The first and most important is subject safety. Administration of an overly large dose of allergen has the potential to produce acute severe local or systemic allergic effects.

The second concern is to choose a method that is reproducible. Because it is usually applied as a research tool, the reproducibility required is within-subject repeatability; the need for between-subject reproducibility, which is important for diagnostic challenges, such as methacholine and histamine, is less important.

Allergen concentrations and selection of a starting concentration

Allergen bronchoprovocation tests are conducted through the administration of progressive serial concentrations (or doses) of the chosen aeroallergen at regular intervals until a given decrease in FEV_1 (eg, 20%; ie, the EAR) is achieved. Concentrations (or doses) of allergen for inhalation are made available in a geometric progression, most often doubling concentrations.[19-21] Some investigators have used half-log[22] (approximately 3.2-fold) or 4-fold[23,24] dose step-ups. Even 10-fold dose step-ups have occasionally been used,[17,25] but this is not recommended for safety reasons. The selection of the starting concentration for the initial allergen challenge, which is frequently referred to as a screening challenge, is based on safety. In the past, investigators have used the weakest of the available allergen concentrations producing a discernible (2-3 mm) wheal on skin prick testing as the starting concentration.[26] Although this is safe, it creates the potential for a long challenge procedure. Several investigators have documented that the allergen-induced EAR is dependent on the level of the baseline nonspecific AHR (generally assessed with direct stimuli, either histamine or methacholine)[20] and allergen-specific IgE levels, which are generally assessed by using skin prick test end point dilution titration (lowest concentration producing a 2- to 3-mm wheal),[27-30] although theoretically, serology (RAST) could be used.[29]

A formula to predict the allergen concentration required to produce an EAR (ie, allergen concentration causing a decrease in FEV_1 of 20% from baseline) has been developed based on skin prick test end point and PC_{20}(histamine/methacholine) values.[5] A prediction equation in house dust mite–sensitive asthmatic patients with only PC_{20}(histamine/methacholine) has also been shown to be effective (Table II).[20] These predictions are accurate to within 2 (80% to 81%) or 3 (92% to 94%) doubling concentrations, and hence it is safe to start 3 concentrations at less than the APC_{20}.

There are a number of points to make about these prediction equations. First and most important, the prediction equations are method specific. For the derivation of the equations described above, histamine/methacholine and allergen inhalation were conducted by using identical methods, namely 2-minute tidal breathing from a jet nebulizer (eg, Wright nebulizer; Roxon Meditech, Montreal, Quebec, Canada; Bennett Twin nebulizer, Puritan Bennett Corporation, Carlsbad, Calif; or a DeVilbiss 646 jet nebulizer, Somerset, Pa) with an output of 0.13 mL/min.[5,20,31] Second, the skin test end point might not be particularly reproducible and can be investigator dependent. Third, use of an indirect measurement of airway responsiveness (AMP and mannitol)[32] has not been assessed in this regard. Intuitively, it might seem an attractive alternative because indirect airway responsiveness correlates better with airway inflammation.[12,32] However, many of the patients with well-controlled mild asthma have negative indirect challenge results, and these are generally the target subjects for research allergen challenges. The choice and preparation of inhaled allergens are detailed in the text in this article's Online Repository. Using flow cytometry to measure CD63 upregulation on CD203c-identified basophils has been tried as another approach to determining allergen sensitivity to predict the EAR.[33] Recently, in a large study including children with house dust mite allergy (5-18 years), apart from PC_{20}(methacholine) and serum specific IgE levels, exhaled nitric oxide (eNO) was found to be a good predictor of the EAR.[34]

Challenge methods

There are 3 validated methods for inhaled allergen challenge under experimental conditions (2 "high-dose" challenges):

1. the titrated dose step-up or incremental dose[5,20]
2. a single-bolus dose method[14] and
3. the repeated low-dose challenge protocol.[15]

Incremental allergen dose challenge

In all studies a baseline or screening allergen challenge is initially done to identify the (cumulative) dose producing an EAR

TABLE I. Subject inclusion/exclusion criteria for allergen challenge and asthma stability criteria at repeat challenges

Inclusion criteria
Age: 18-55 y; overall good health (especially no cardiovascular problems or chronic sinusitis); good understanding of asthma, precipitating factors, and medication use; willing to comply with the protocol's rules and capable of performing spirometry well
Well-defined, physician-diagnosed, clinically stable asthma; baseline (ie, preallergen) FEV_1 ≥70% of predicted value at screening and ≥65% or ≥2 L during subsequent study periods; baseline PC_{20}(methacholine) or histamine <16 mg/mL at screening
Able to refrain from controller medications, as defined in Table E1, without clinically relevant asthma worsening throughout the study; stable, infrequent, as-required short-acting rescue medication use only; stable FEV_1 and PC_{20}(methacholine or histamine) criteria
Demonstrated allergy (skin prick test or blood test) to aeroallergens with a clinical relationship between allergen exposure and asthma symptoms (see the text in this article's Online Repository for the choice of inhaled allergen); if multisensitized and symptomatic, the allergen challenge should be performed outside the relevant allergen exposure or season
No relevant bronchoconstriction (ie, ≤10% decrease in FEV_1 from baseline) 10 min after inhalation of the allergen's diluent
Current nonsmokers (stopped at ≥6-12 mo ago; ≤10 pack years)
No caffeine-containing drinks or products within at least 8 h of bronchoprovocation testing
No viral or other respiratory tract infections within ≥4 wk before challenge
Exclusion criteria
Bronchoconstriction at screening (baseline, preallergen FEV_1 <70% of predicted or <2 L) or at repeat challenges (study periods; baseline FEV_1 <65% of predicted or <2 L)
Spirometry-induced bronchoconstriction (ie, <2 baseline FEV_1 measurements of 8 attempts within 5%)
Recent (<4 wk) viral respiratory tract infections
Recent (<1 y) hospital admission for asthma or frequent asthma exacerbations requiring hospital admission or oral corticosteroids
Recent major surgery; history of angina pectoris, myocardial infarction, or compromised left ventricular function; *any* history of cerebrovascular accident, arterial aneurysm, seizures, untreated hypertension, active hyperthyroidism, active or chronic infection, immunologic disorder, cancer, pregnancy or lactation, severe drug allergy, or history of anaphylaxis
Inability to comply with procedures related to allergen challenge
Stability criteria for repeat challenge
Baseline FEV_1 and PC_{20}(methacholine or histamine) should be measured at the same time of day during the entire study (ie, within a timeframe of 2-3 h)
Baseline (ie, preallergen) FEV_1 in study period I in a crossover study should remain within 10% of screening (and ≥65% of predicted), and in subsequent study periods all preallergen baseline values should remain within 10% of the preallergen value in study period I
No significant bronchoconstriction within 10 min after inhalation of the diluent (≤10% decrease from baseline)
PC_{20}(methacholine or histamine) should be performed (preferably 24 h) before allergen; in a crossover study, study period I, preallergen PC_{20}(methacholine or histamine) should remain within 1 doubling concentration of screening and in the subsequent study periods; preallergen PC_{20}(methacholine or histamine) should remain within 1 doubling concentration of the preallergen value of study period I; if not, postpone the allergen challenge by 1-4 wk, depending on the cause

Subject suitability to undergo an allergen challenge will depend on individual characteristics, as judged by a qualified and experienced physician.

with a 20% or greater FEV_1 decrease, to document whether there is also an LAR, and to determine the allergen doses to be inhaled during subsequent allergen challenges in the study. Increasing concentrations (generally doubling) of allergen are inhaled at approximately 12-minute intervals (ie, between the start of one and the start of the next inhalation), with spirometry measured in duplicate 10 minutes after each consecutive allergen dose. A diluent challenge day has often been used in the past[19] to differentiate the allergen-induced EAR from a nonspecific irritant effect and the LAR from spontaneous fluctuations in airway caliber. For research purposes, this can be considered optional, and presently, the diluent day has been substituted by a single diluent inhalation before challenge. The objective is to (safely) administer the same (cumulative) concentrations of allergen in the same fashion throughout the study under each treatment condition.

In crossover studies (ie, repeat challenges under different treatment conditions) it is common to administer the last 3 (highest) allergen concentrations that were administered during screening. This allows administration of a dose that generally approaches 90% of the total amount of allergen administered during screening. It is possible that the (slightly) lower total dose of allergen administered in this fashion could reduce the clinically important late sequelae (eg, an LAR, airway eosinophilia, and allergen-induced AHR), but this has not been studied. Despite ensuring subject stability (baseline FEV_1 and baseline AHR), even in experienced hands, the limit of repeatability is ±1 doubling allergen concentration.[35] When a subject responds at a lower concentration of allergen, it might not be possible to safely administer all 3 concentrations during repeat challenges. If a decrease in FEV_1 of greater than 20% is already achieved at the second of 3 allergen concentrations during the first period of a crossover study, it might be appropriate to proceed with the study using a similar dose (ie, the same 2 allergen concentrations in the next study period or periods). However, should this happen in a crossover study at the second or later study period, it will be difficult to compare data across the study periods for this subject because protection studies require the same allergen doses for active and placebo treatment. For parallel studies, the screening challenge can be used to ensure subjects meet the inclusion criteria and to ensure that the groups were reasonably matched.

The choice of nebulizer (jet nebulizer vs dosimeter) and choice of method of inhalation (tidal breathing vs counted deep breaths) are only important in that the method should be (within-subject) reproducible. The largest experience is with the 2-minute tidal breathing method using a Wright nebulizer (Roxon Meditech) with an output of 0.13 mL/min. However, any durable, calibratable, and reproducible jet nebulizer (eg, DeVilbiss 646)[20] can be used, provided it is run under identical conditions for each challenge.[20,36] A counted breath dosimeter can also be used for incremental dose challenges.

Single-bolus allergen dose challenge

For the single-bolus challenge method, the baseline screening allergen challenge is performed in a titrated dose step-up manner

TABLE II. Formulae used to predict the allergen-induced early response (ie, allergen concentration causing a decrease in FEV_1 of 20% from baseline) from AHR to PC_{20}(histamine or methacholine) with or without skin prick test[5,17]

Predicting allergen-induced EAR (APC_{20}) from AHR (PC_{20}[histamine] or PC_{20}[methacholine]) and titrated SS[5]: $\log_{10}(APC_{20}) = 0.68 \times \log_{10}(PC_{20}[\text{histamine or methacholine}] \times SS)$
Predicting house dust mite*–induced EAR (APC_{20}) from AHR (PC_{20}[methacholine] only)[20]: $\log_{10}(APC_{20}) = -0.902 + 0.741 \times \log_{10}(PC_{20}[\text{methacholine}])$

APC_{20}, Allergen PC_{20} predicted value (predicted provocative allergen dose required to produce a 20% decrease from baseline FEV_1); *SS*, skin prick test end point.
*Equation by Ravensberg et al based on allergen challenges with inhaled house dust mite extract only.[20]

to identify the cumulative dose of allergen required to produce an EAR.[14,17] Subsequent repeat challenges are then performed with this single dose of allergen. Traditionally, this method has generally been performed by using counted deep breath inhalations from a dosimeter method, although theoretically, tidal breathing could also be used. The single-dose bolus has 2 attractive features. The first is a reduction of the challenge time by approximately 30 minutes. More importantly, the second is the guarantee of being able to administer the same dose of allergen on each occasion. However, a safety concern is that the administration of a single high dose of allergen has the potential to produce a greater EAR than the same dose of allergen administered incrementally over 20 to 30 or more minutes during screening. Many investigators believe that the extra 30 minutes involved in a 3-dose incremental step-up is a small price to pay for the subject's comfort and safety.

Incremental versus single-bolus method

Both the incremental titrated dose and single-bolus challenge methods produce comparable airway responses.[14,17] Although both methods are validated, there are some differences that might have important implications. The incremental allergen challenge features a built-in safety, allowing the full challenge to be aborted in case of an unexpectedly large response to an earlier dose. Deep-breath inhalations during the single bolus method might predispose to inaccurate dosing because of potential variation in the respiratory maneuvers, the possibility of cough during the procedure, and the potential influence on lung function outcome of deep breath–induced changes in airway caliber. If the allergen challenge outcomes are being compared between 2 or more interventions, it is necessary to choose a delivery method to minimize variability of the inhaled allergen dose. Whichever method is applied, prechallenge asthma stability should be warranted by the subject's history and stable baseline (FEV_1 and PC_{20}) values (Table I).

Repeated low-dose allergen challenge

Inhalation of relatively high doses of allergen during a single brief period might not mimic natural allergen exposure. Smaller doses of allergen titrated to cause minimal bronchoconstriction and administered once daily over 1 or 2 weeks might come closer to mimicking natural allergen exposure.[37]

Protocols have been validated for inhalations of low doses of allergen producing a 5% FEV_1 decrease from baseline and repeated for 5, 7, or 10 days.[38-41] This procedure induces airway eosinophilia[38,41-45] and increases eNO levels,[40,41,43,45] and AHR[15,42,44,46,47] probably more pronouncedly than that seen after a single high-dose allergen challenge inducing a dual asthmatic response.[38,40,41,43,44] Notably, the increase in AHR occurs in the relative absence of asthma symptoms. Therefore this challenge model has received interest as a valuable tool to investigate early events of importance for the development of symptomatic and possibly persistent asthma. In addition, this model has been useful in evaluating drug effects, particularly for inhaled corticosteroids.[43-45] However, it is not as widely used as the high-dose challenges, likely because of the large number of subject visits required.

EXPRESSION AND ANALYSIS OF THE ALLERGEN-INDUCED AIRWAY RESPONSES

High-dose allergen challenges

There are several airway responses that can be observed after allergen inhalation (Fig 2 and see the text and Fig E1 in this article's Online Repository). When subjects are challenged with inhaled allergen to which they have been sensitized, cross-linking of antigen/IgE and IgE receptors induces immediate release of bronchoconstriction mediators (eg, histamine, leukotrienes, and prostaglandins) from airway mast cells and basophils. The EAR usually occurs within 10 minutes and resolves within 2 hours of allergen inhalation. The EAR is usually defined as a decrease in FEV_1 of at least 20% from baseline. Generally, the decrease in FEV_1 during the EAR is maximal 10 to 30 minutes after allergen,[48] and its magnitude depends on baseline nonspecific AHR, the serum specific IgE level, and the dose of allergen inhaled.[31] The EAR can be expressed as the maximum percentage decrease in FEV_1 from the preallergen (usually postdiluent) baseline or as the area under the curve (AUC) of the percentage decrease in FEV_1 versus time during the first 2 hours after challenge ($AUC_{0\text{-}2h}$).

Approximately 50% to 70% of subjects challenged with inhaled allergen have an isolated EAR that resolves within approximately 2 hours with no further bronchoconstriction or increased nonspecific AHR,[6] whereas the other 50% have an LAR after resolution of the EAR.[48] In a minority, usually the more hyperresponsive asthmatic patients, the EAR does not fully resolve to baseline values and directly progresses into an LAR (unpublished data). The LAR is usually characterized by a decrease in FEV_1 of 15% or greater from baseline, occurring between 3 and 7 or more hours after allergen, with a maximum between 8 and 12 hours after challenge. Its main characteristics comprise the increase in T_H2 cell–driven airway inflammation with eosinophils as key effector cells and the associated nonspecific AHR (Fig 2).[9,49] Furthermore, animal models provided ample evidence for the involvement of sensory and cholinergic nerves in the pathophysiology of the LAR,[50] although in human subjects thus far, tachykinin receptor antagonists did not modulate allergen-induced airway[51] responses. The allergen-induced AHR usually develops 3 hours after allergen and can last for 2 to 3 weeks (see Fig E1).[6,48,52]

The magnitude of the LAR within subjects is closely related to the magnitude of the EAR, which implies it develops at least in part due to IgE-mediated pathways. Yet another observed response is an isolated LAR, which has been observed after inhalation of peptides[53] that cannot activate IgE-mediated pathways.

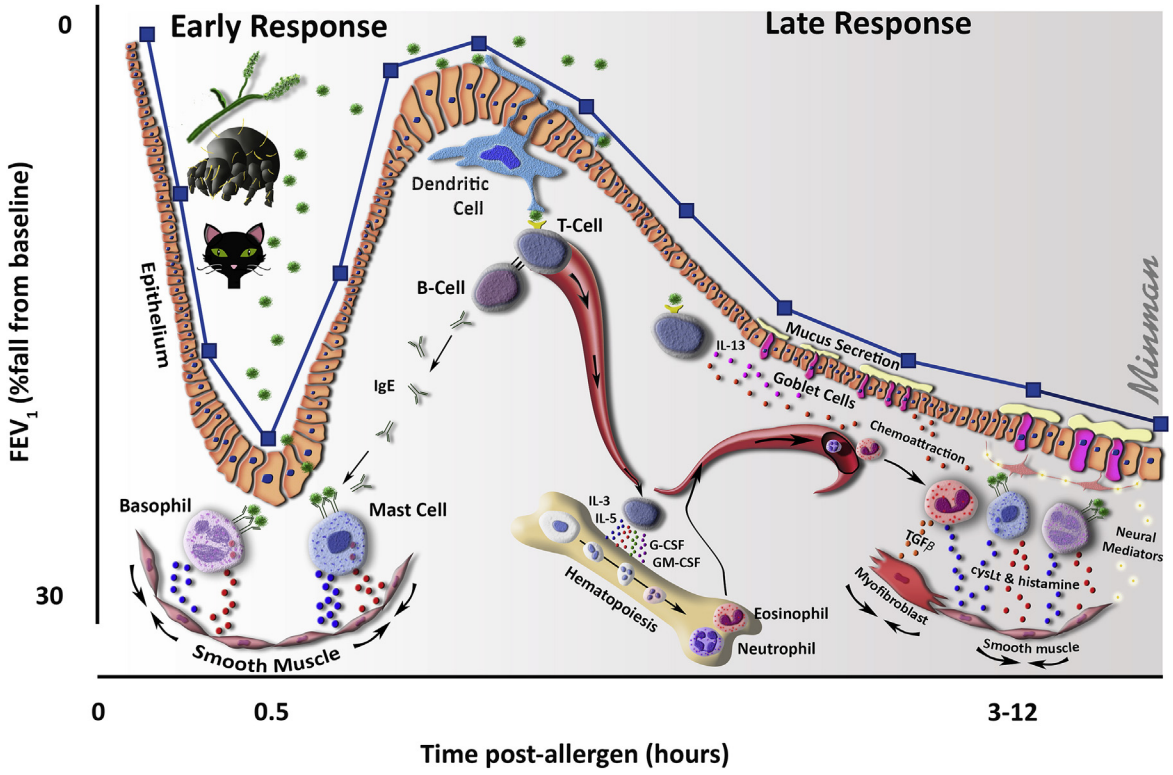

FIG 2. EAR and LAR after high-dose inhaled allergen challenge.

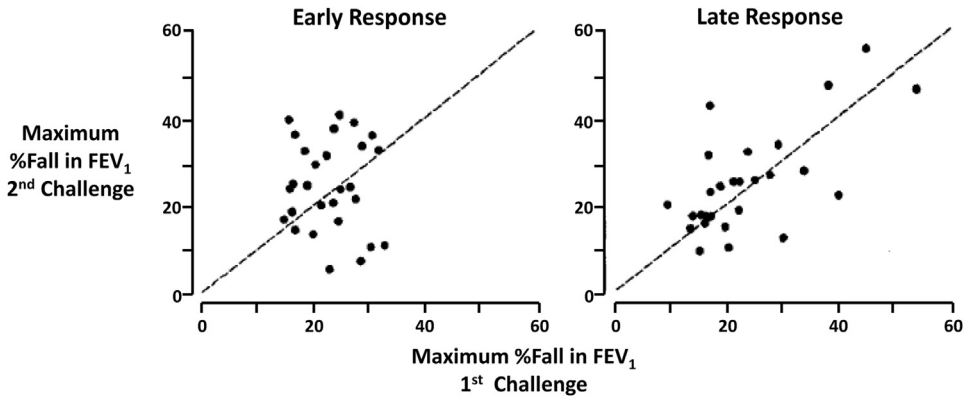

FIG 3. The relationship between the maximal decrease in FEV_1 from baseline during the EAR *(left)* and the LAR *(right)* after 2 consecutive allergen challenges performed in the same subjects (n = 28). Reproduced with permission from Inman et al.[35]

As with the EAR, the LAR is expressed as the maximum percentage decrease in FEV_1 or the AUC of the percentage decrease in FEV_1 versus time, usually from 3 hours after challenge onward (eg, $AUC_{3\text{-}8h}$). Comparisons of maximum percentage decreases in FEV_1 and AUC appear to be equally reproducible.[35,49] However, the AUC is more widely accepted because it is less sensitive to single outlier measurements than the maximum percentage decrease in FEV_1.[18] It is important to standardize the length of time within which FEV_1 measurements are collected because the FEV_1 can continue to change over time. The length of recording time must also be kept constant to correctly compare AUC values within and between subjects.

Other airway measurements that can be used to assess the effects of inhaled allergen include the FEV_1 measurement at 24 hours after allergen,[54] although this is not as sensitive as the LAR because the change from baseline is often considerably smaller and might be confounded by intercurrent use of rescue medication. The allergen-induced shift in AHR is also used to indicate the effects of allergen inhalation. At 24 hours after challenge, the PC_{20}(methacholine or histamine) is observed to decrease by 1 to 2 doubling doses.[9,36,49,55] As such, comparing the Δ PC_{20}(methacholine or histamine) before with that after allergen provides another useful outcome,[18] although it is not as sensitive as the LAR because of larger variability in measurements.[56]

Assessments of the components of airway inflammation, such as sputum eosinophil counts and soluble mediator levels, or surrogate markers, such as eNO levels, have shown consistent and

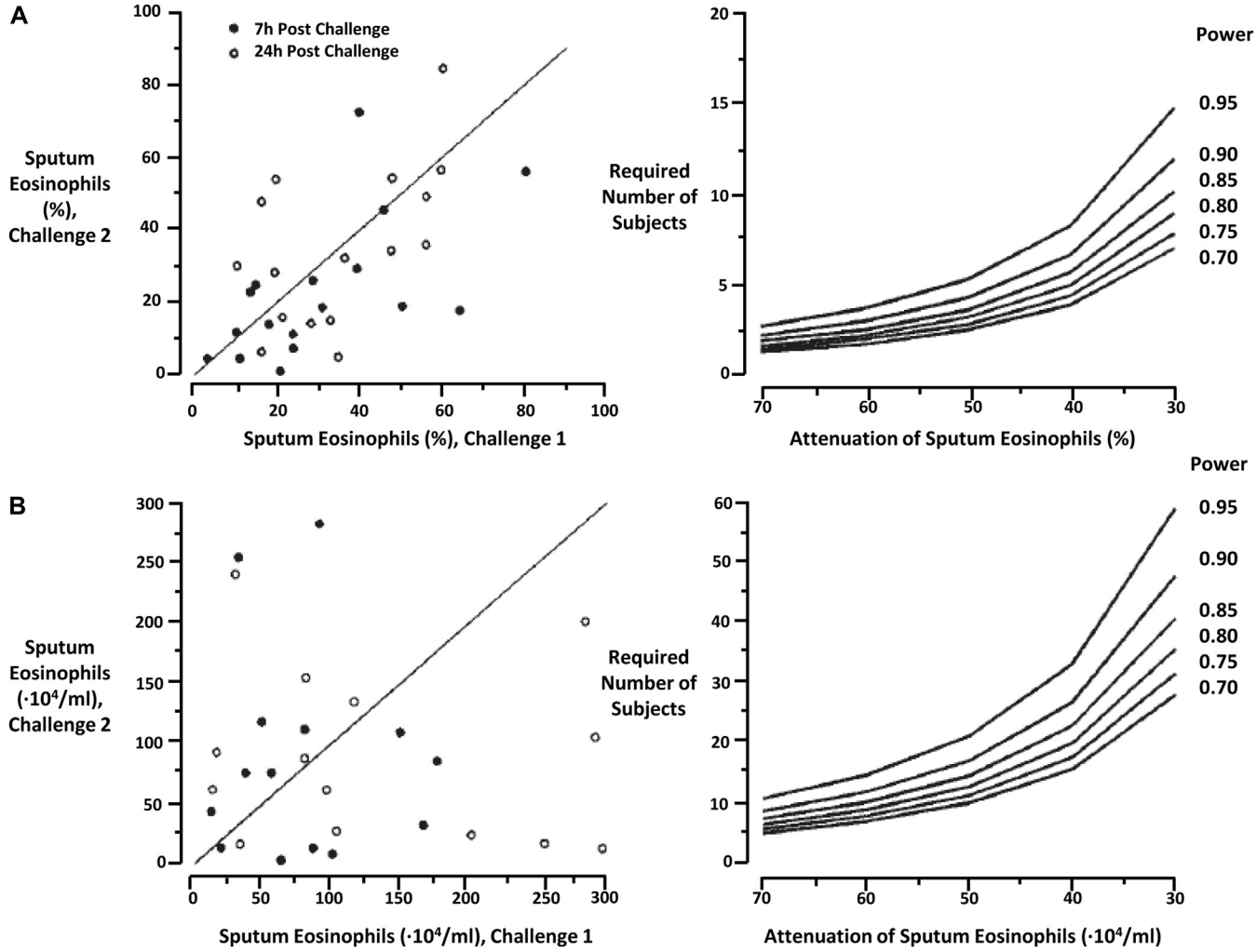

FIG 4. A, Relationship between the AUC during the LAR after 2 subsequent allergen challenges performed in the same subjects at least 3 weeks apart (n = 17, *left*) and power curves allowing estimation of sample size for expected attenuation of the LAR AUC (in a crossover study, *right*). Reproduced with permission from Gauvreau et al.[49] **B,** Relationship between the percentage of sputum eosinophils at 7 hours *(solid circles)* and 24 hours *(open circles)* after 2 consecutive allergen challenges performed in the same subjects at least 3 weeks apart (n = 17, *left*) and derived power curves allowing estimation of sample size for expected attenuation of the allergen-induced percentage sputum eosinophils (crossover study, *right*). Reproduced with permission from Gauvreau et al.[49]

reproducible increases after high-dose allergen challenges (see Fig E2).[9,36,49,57,58]

Low-dose allergen challenges

The repeated low-dose allergen challenge primarily allows evaluation of allergen-induced changes in AHR and airway inflammation. The increases in AHR can be analyzed as ΔPC_{20}(methacholine) or PD_{20}(methacholine) (before-after challenge). In a diluent-controlled evaluation of this method, sputum eosinophil counts and IL-5 and eosinophil cationic protein levels were found to increase after allergen.[38] This increase in sputum eosinophil counts has been reproduced in other low-dose allergen challenge studies.[41-45] In addition, 2 studies reported increases in eosinophil counts and eosinophil cationic protein levels in bronchoalveolar lavage fluid.[42] Similarly, reproducible increases in daily measurements of eNO levels have been found in several studies.[40,41,43,45] Other evaluable outcomes include daily recordings of changes in asthma symptoms, rescue medication use, and FEV_1.

Repeatability, reproducibility, and within-subject variability

The repeatability and reproducibility of the allergen-induced airway responses, markers of airway inflammation, and nonspecific AHR have been extensively studied with the 2-minute tidal breathing method, applying incremental allergen doses.[9,35,49,59] These studies demonstrated excellent within-subject repeatability of the EAR and LAR, both when analyzed as the respective maximal percentage decrease in FEV_1 from baseline and as the area under the time-response curve (Fig 3).[35,49] Also, reproducible changes in allergen-induced AHR and airway inflammatory markers have been described.[9,49,59] Although the percentage of sputum eosinophils proved a reproducible marker of the allergen-induced airway inflammation, the absolute eosinophil

TABLE III. Predictive value of allergen challenge in clinical drug development of currently registered and novel asthma-controlling agents

True-positive results	True-negative results	False-positive results	False-negative results
Conventional ICSs	Esterase-sensitive steroids	Anti-CD11a hMAb	
Oral corticosteroids (prednisone)	PAF antagonists	Inhaled PGE_2	
Combination ICS/LABA	Inhaled leukotriene modulators	Antihistamines	
Cromoglycate	Inhaled anti-IgE hMAb		
Oral leukotriene modulators	Thromboxane antagonists		
Subcutaneous anti-IgE hMAb	Ca^{2+} channel blockers		
Allergen-specific immunotherapy	Nitric oxide synthase inhibitors		

Modified from Gauvreau et al[9] and Boulet et al.[11]

False-negative results, drugs ineffective against the LAR but clinically effective; *False-positive results*, drugs effective against the LAR without clinical efficacy; *True-negative results*, drugs ineffective against the LAR and in clinical applications; *True-positive results*, drugs effective against the allergen-induced LAR with subsequent proved clinical efficacy.

hMAb, Humanized mAb; *ICSs*, inhaled corticosteroids; *LABA*, long-acting β_2-agonists; *PAF*, platelet-activating factor; *PGE*, prostaglandin E.

numbers appeared less reproducible because of methodological factors.[49] By using sensitive detection techniques, the changes in T_H2-derived cytokine levels recovered from sputum after allergen challenge showed overall good within-subject repeatability.[59]

This challenge method shows an excellent within-subject repeatability, and hence in crossover studies only limited numbers of subjects are required to show a statistically significant expected attenuation in these allergen-induced parameters (Fig 4, *A* and *B*).[35,49] If several outcome measures (eg, sputum cell differentials and soluble markers) are being explored within an allergen challenge study, the sample size needs to be based on the outcome parameter with the largest variability.

Although there is no formal study on the repeatability of the outcome variables after repeated low-dose allergen challenge, the increase in AHR is a very consistent and reproducible finding across almost all the different protocols.[15,42,44,46,47]

REPEAT CHALLENGES AND RECOVERY

High-dose allergen challenges

Care must be taken to ensure sufficient recovery from a previous allergen challenge before performing subsequent challenges in (crossover) studies. Any interfering factors, including seasonal allergies or viral respiratory tract infections, need to be excluded (see the text in this article's Online Repository at www.jacionline.org and Table I). In most subjects with dual responses, allergen-induced AHR and airway inflammation levels have not returned to baseline within 7 days after allergen (see Fig E2),[9] whereas in the majority these measurements are back to baseline values by 14 days after allergen (unpublished data). A minimum of a 14-day recovery between consecutive challenges has been successfully implemented in many crossover studies[36,55,57,60-69] and parallel-group studies with multiple challenges.[21,70-72] By measuring baseline spirometry and AHR before subsequent allergen challenges (Table I, stability criteria), the infrequent number of subjects with AHR or FEV_1 that does not return to baseline values by 2 weeks are simply given an additional 1 to 2 weeks before the next challenge. Although it is unlikely that a 2-week washout period is sufficient for all components of the immune response to return to baseline levels after inhaled allergen challenge, including allergen-specific IgE levels,[73] T_H2 cytokine levels,[59,74] and plasma cell[75] and structural cell[76] counts, the sample size calculations for the LAR as the primary outcome have been determined by using data collected from challenges at least 2 weeks apart,[35,77] and thus it is possible to power studies appropriately for the LAR regardless of these caveats.

Low-dose allergen challenges

In a placebo-controlled, crossover, 3-period intervention study using a 7-day exposure protocol, there were no period or carryover effects for PD_{20}(methacholine) or for eNO, with a 15-day washout between study periods.[43] With a 5-day protocol,[38] the mean reduction in PC_{20}(methacholine) level was reversed 3 days after the last allergen dose, as were sputum eosinophil counts and their activation markers. No formal data exist with regard to washout for eNO. Because the magnitude of eosinophilia amounts to the same as that observed after high-dose allergen challenge, it might be safe to recommend a similar washout of at least 2 weeks. The number of allergen exposure days could affect the recovery time.

APPLICATIONS AND CONCLUSIONS

Allergen challenge is primarily a research tool and not a standard test for common clinical practice.[78] An important exception is in the investigation of occupational asthma and rhinitis, in which a specific challenge with allergens found at the workplace (both high-molecular-mass substances, such as flour and enzymes, and low-molecular-mass agents, such as diisocyanates) might help to confirm the diagnosis.[79-81] In research settings a dual airway response (ie, both an EAR and an LAR) is generally considered more useful because it more closely reflects naturally occurring asthma. Especially if a dual response is combined with airway samplings, allergen challenge offers an integral model of allergic asthma, allowing the study of links between acute and more chronic allergen-induced inflammatory events and physiologic sequelae within the airways and their responsiveness to new and existing (targeted) asthma therapies.[11-13]

For most established asthma controller therapies, inhibition of allergen-induced increases in sputum eosinophil counts has been shown to be associated with LAR inhibition.[54,59,60,69] However, a discrepancy between sputum eosinophil counts and the LAR was found after treatment with an anti–IL-5 mAb,[71] underscoring the complexity of the physiology of the LAR and potential involvement of nonimmunologic mechanisms. When anti–IL-5 treatment was applied in a subset of asthmatic subjects with therapy-resistant sputum eosinophilia, clinical efficacy was

achieved.[82,83] Allergen-induced eNO levels have not always adequately reflected drug efficacy observed on measurements of LAR.[58] However, the lack of clinical efficacy of inhaled NG-nitro-l-arginine methyl ester, a nonselective nitric oxide synthase inhibitor, on allergen-induced airway responses[84,85] could have been caused simply by its mechanism blocking nitric oxide, which is a bronchodilating agent.[86]

Generally, inhibition of the LAR and associated sequelae in early efficacy studies has proved to be an effective predictor of the actual clinical efficacy of new and existing asthma-controlling agents (Table III).[11,12] Overall, inhaled corticosteroids showed the most complete inhibition of the allergen-induced late-phase sequelae and have proved superior clinical efficacy.[54,59,60,63,87-89] These drugs act at several points upstream of the inflammatory cascade and are generally less specific, whereas targeted therapies generally need to show at least 50% protection against the LAR (and/or associated sequelae) to prove clinical efficacy in a specific phenotype,[67,70] although the magnitude of the actual clinical benefit cannot be extrapolated from its effects on allergen response. Apart from its overall good positive predictive value for drug efficacy in (allergic) asthmatic subjects, allergen challenge also showed an excellent negative predictive value.[11,12] In the latter context it should be noted that this obviously only applies for agents administered at an effective dose regimen failing to show (sufficient) efficacy against any of the allergen-induced late sequelae.

Although bronchodilator agents can (partly) inhibit allergen-induced airway responses, their activity is mainly based on functional antagonism, which is in line with their limited efficacy on airway inflammation and overall clinical efficacy when applied as monotherapy in asthmatic subjects.[90,91]

In addition to the general limitations that apply to disease models, allergen challenge mainly reflects aspects of the allergic asthma phenotype. Furthermore, the strict subject selection criteria and the procedure itself, in combination with airway sampling, might be demanding for both the subject and the investigator. Alternatively, if safely and properly performed, allergen challenge offers a valuable tool for assessing a drug's clinical efficacy in a small sample size of subjects.

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HISTORICAL ASPECTS OF ALLERGEN BRONCHOPROVOCATION TESTS

In 1873, Charles Blackley[E1] described the first challenges with grass pollen performed on himself. Fifty year later, Stevens[E2] published on the respiratory and cutaneous effects of allergen exposure. Nebulized aqueous solutions were administered, and only early airway responses were recorded by using symptoms, signs, and simple pulmonary function tests, such as vital capacity,[E3-E5] before additional measures of airway obstruction could be added, according to the suggestion of Tiffeneau.[E6] The FEV_1 was standardized in 1957[E7] and remains a key parameter of the airway response to inhaled allergen.

Initial observations mainly reported on EARs,[E3-E5] although Blackley[E1] already had described what would now be recognized as an LAR. In the early 1950s, Herxheimer[E8-E10] performed pivotal allergen challenge studies and described the LAR in which he attributed recovery after the EAR to the bronchodilator administered after the EAR. The biphasic nature of the allergen-induced response occurring in approximately 50% of asthmatic subjects was recognized in the late 1960s.[E11,E12] In the 1970s, research groups led by Orie in the Netherlands[E13,E14] and by Pepys in the United Kingdom[E15-E17] applied these features to study the pathophysiology of asthma and the effects of asthma medications. The commonly observed increases in nonspecific AHR after both natural and laboratory allergen exposure were initially described by Altounyan[E18] and Hargreave et al.[E19] The latter led to a series of studies reported by Cockcroft et al,[E20,E21] defining the links between allergen-induced LAR and subsequent AHR (Fig E1).[E22,E23] The next step in refining the analysis of allergen-induced airway responses was the documentation of allergen-induced airway inflammation, which was first reported by De Monchy et al[E24] in 1985 using bronchoalveolar lavage. Noninvasive airway sampling methods were subsequently developed (Fig E2).[E25-E27]

CHOICE OF INHALED ALLERGEN

The choice of the sensitizing allergen extract for inhalation is based on the largest skin wheal elicited by skin prick testing or the highest serum specific IgE level[E28-E30] matched with a history of symptoms of wheeze or chest tightness after exposure. Sensitizing allergens vary geographically. For example, house dust mite allergies are common in areas with humid conditions and less common in dry regions where dust mites do not thrive. In addition, certain trees, weeds, and grasses are native to specific areas. The inhaled allergen dilutions should be prepared from commercially available standardized allergen extracts and should be void of endotoxin contamination.[E31] In some countries allergen extracts that have been prepared for skin testing can be inhaled with approval of local ethics committees; in the European Union good manufacturing practice certificate is needed, whereas in the United States, each allergen extract to be used for inhalation is treated as an investigational new drug and must receive US Food and Drug Administration approval for inhaled delivery.

It has been noted that extracts can elicit stronger airway responses compared with purified allergens,[E32] likely through activation of non–IgE-mediated pathways.[E33-E36] Furthermore, the magnitude of the LAR has been found to be related to specific allergens because house dust mite has been shown to provide a greater LAR in the same (sensitized) subjects than grass pollen.[E37] Consequently, it is important to choose the appropriate allergen.

Although it is recognized that some allergens can induce responses through non–IgE-mediated mechanisms, it is not known whether this difference is reflected in cellular infiltrates, AHR, and/or inflammation at different sites of the airways (eg, small vs large airways).

Where mechanisms of allergen-induced responses are under investigation, it would be preferable to challenge all subjects with the same allergen to avoid the possibility of variability in responses because of stimulation of protease-activated receptors and other non–IgE-mediated proinflammatory pathways. However, limiting challenges to just 1 allergen can make a study very complicated and lengthy. Within an interventional clinical trial, it is possible to use different allergens for different subjects, and estimates of appropriate sample sizes have been calculated by using this approach.[E38,E39] However, it is essential to use the same allergen at the same cumulative dose for the same subject during trials in which patients serve as their own control subjects (ie, crossover design).

ALLERGEN PREPARATION

Most formulations of allergen extracts are stable for more than 1 year when refrigerated at 6°C.[E40,E41] Administering the same dose of allergen can be accomplished by either preparing fresh allergen titrations from the same batch on the day of challenge or by preparing a large quantity of the required dose and subsequently freezing sufficient aliquots for the entire study. Freezing has the advantage of avoiding day-to-day variability in preparation but is dependent on each frozen aliquot to undergo the same number of freeze/thaw cycles because extracts can lose biological activity after being frozen.[E42] It is important for allergen extracts to be diluted carefully with the allergen's diluent by experienced laboratory personnel using sterile techniques and the manufacturer's recommendations. Likewise, allergen dilutions should be allowed to reach room temperature before inhalation.

TIMING OF ALLERGEN CHALLENGES

If multiple allergen challenges are to be conducted, recruitment of pollen-sensitive subjects must take place such that the entire study can be completed before the beginning of a pollen season. This is because nonspecific AHR (PC_{20}[methacholine or histamine]) is the main determinant of the allergen-induced EAR[E43,E44] and is known to shift after exposure to allergen[E20] or (viral) respiratory tract infections[E45] and thus can cause exaggerated airway responses to a set dose of allergen. Even if subjects are tested outside of a pollen season, it is possible that exposure to other sensitizing allergens, such as cat or house dust mite, can affect their level of nonspecific AHR. Therefore it is essential for AHR to be within baseline values before administering allergen. This can be effectively confirmed by demonstration of PC_{20}(methacholine or histamine) within 1 doubling concentration of baseline at 24 hours before allergen. It is also recommended that FEV_1 is within 10% of the baseline value (see also Table I).

AIRWAY RESPONSE IN HIGH-DOSE ALLERGEN CHALLENGE PROTOCOLS

Irrespective of the challenge method, a single inhalation of the allergen's diluent should precede the challenge to help discriminate an actual allergen-induced EAR from a nonspecific decrease in FEV_1. The postdiluent decrease in FEV_1 should not exceed

10% from baseline, and if so, the challenge should be postponed for at least 7 days, depending on the cause of asthma worsening.

The subsequent inhaled allergen dilutions depend on the challenge method applied. In both high-dose allergen challenge protocols, the screening challenge is a dose-finding procedure for the EAR and explorative for the occurrence of the LAR. In the incremental allergen dose challenge, allergen dilutions are derived by using the (modified) Cockcroft formula, adding 3 lower serial doubling dilutions (eg, allergen PC_{20} predicted, 1:8; start challenge at 1:64).[E43,E44] Subsequently, increasing doubling concentrations of allergen are inhaled until an EAR is provoked (defined as a ≥20% decrease in FEV_1 from baseline) or until the highest concentration has been inhaled. All allergen concentrations are inhaled by means of tidal breathing for 2 minutes at approximately 12-minute intervals. At 10 minutes after each allergen concentration, the airway response is measured in duplicates, and the largest, technically adequate FEV_1 is expressed as the percentage decrease from postdiluent baseline. If the FEV_1 decreases by less than 15%, the next concentration will be inhaled. If the FEV_1 decreases to between 15% and 20%, it should be measured again after another 5 minutes, and if by then a decrease in FEV_1 of 20% or greater is reached, no further allergen is inhaled. If the decrease is still between 15% and 20%, the next concentration is inhaled for 1 minute instead of 2 minutes (or until the EAR occurs).

After the provocative allergen dose causing the EAR, the airway response is measured at 10-minute intervals until 1 hour after allergen and subsequently at 90 and 120 minutes, followed by hourly measurements until 7 or more hours after allergen. It is recommended that this monitoring should always be performed because, even in the absence of an EAR, an LAR can still occur. In case of a marked decrease in FEV_1 between time points, FEV_1 should be monitored more frequently. After the last FEV_1 measurements, subjects should receive a bronchodilator until the FEV_1 returns to within 10% of the preallergen baseline value. In all cases subjects can only be sent home with a stable FEV_1, rescue medication, written instructions, and emergency telephone numbers.

In subsequent study periods the same highest doses that previously induced the EAR and LAR at screening will be used in each subject after asthma stability is confirmed (Table I, asthma stability criteria at repeat challenges).

When a single allergen bolus method is used, all subjects will start inhaling the same starting dose during the dose-finding procedure at screening. Depending on the subsequent airway response, this dose will be quadrupled (decrease in FEV_1 <10%), doubled (decrease in FEV_1 between 10% and 15%), or (re)repeated (decrease in FEV_1 between 15% and 20%) until an EAR (decrease in FEV_1 ≥20%) occurs.[E46] Similarly, with the incremental titrated method, FEV_1 will be measured at regular times until 7 or more hours after allergen, and the same safety procedures will follow before dismissal.

In the subsequent study periods the cumulative allergen dose that previously induced the EAR will now be administered as an allergen bolus.[E46]

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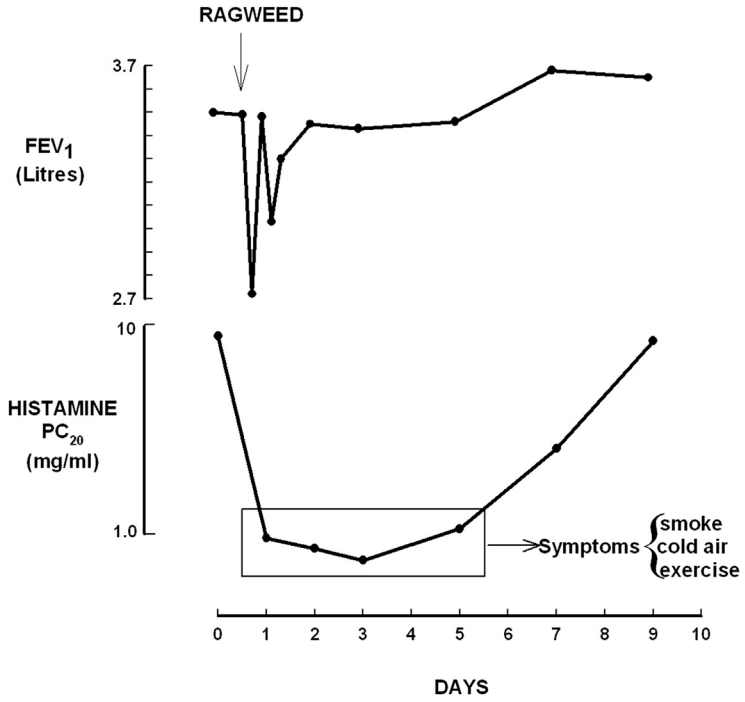

FIG E1. Effect of inhaled allergen challenge on nonspecific airway hyperresponsiveness PC_{20}(histamine). Permission obtained from Cockroft et al.[E21]

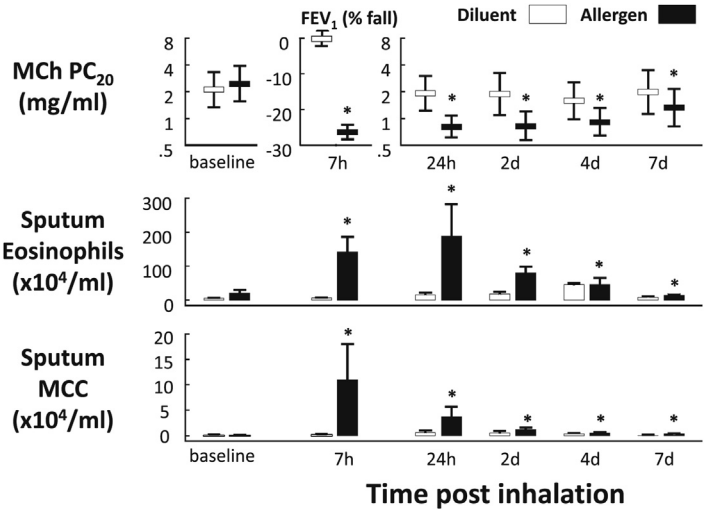

FIG E2. Effect of inhaled allergen challenge on sputum inflammatory markers. Gauvreau et al,[E27] reprinted with permission of the American Thoracic Society. Copyright © 2012 American Thoracic Society.

TABLE E1. Recommended medication withdrawal times before allergen challenge

Medications	Withdrawal time before allergen challenge
Short-acting β_2-agonists	6-8 h
Long-acting β_2-agonists (eg, salmeterol or formoterol)	48 h
Ultra–long-acting β_2-agonists (eg, indacaterol or vilanterol)	72 h
Short-acting anticholinergics (ipratropium)	6-8 h
Long-acting anticholinergics (eg, tiotropium)	72 h
Inhaled or intranasal corticosteroids	4 wk
Oral corticosteroids	8 wk
Short-acting antihistamines	1 wk
Leukotriene modulators	2 wk
Phosphodiesterase inhibitors	2 wk
Anti-IgE	6 mo
Antibiotics (indication: respiratory tract infection)	4 wk
Antibiotics (other indications)	2 wk
Vaccines altering T helper cells	3 mo
Allergen-specific immunotherapy	Contraindicated if applied with the provoking allergen
Aspirin and NSAIDs	At least 7 d, depending on duration of effect

In general, the recommended prechallenge withdrawal times for medications depend on the combination of their duration of action and, where applicable, the (estimated) duration of their anti-inflammatory effects.
NSAIDs, Nonsteroidal anti-inflammatory drugs.