The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper

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Clinical and Experimental Allergy Reviews

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Summary
Allergic rhinitis (AR) is a common condition with quality of life and economic implications for those affected. Numerous studies have attempted to evaluate treatments for rhinitis, seeking clinically meaningful efficacy and safety results to enable evidence-based treatment decisions. Traditional studies of medications for AR are hampered by many confounding environmental factors as well as suboptimal medication compliance. They are also an unsuitable setting for determination of precise pharmacodynamic properties of medications, including onset and duration of action. Allergen challenge chambers (ACCs) were developed to provide predetermined, controlled allergen levels and to limit variables inherent in traditional studies. An ACC hosts a number of allergen-sensitive subjects who may receive either medication or placebo in a closed environment regulated for temperature, humidity and other variables. Subjects’ allergic responses are monitored using subjective and objective assessments throughout the study, and the resultant information contributes significantly to the clinical profile of a medication. This consensus paper provides an in-depth review of the role of ACCs as a means to evaluate treatments in AR, and concludes that ACC trials fulfil an important supportive role in the assessment of anti-allergic medication.

Keywords allergen challenge chamber, allergic rhinitis, consensus, efficacy, treatment evaluation

Allergic rhinitis: pathophysiology and symptom management

Allergic rhinitis (AR) is an upper respiratory disorder that affects at least 20% of the population of Western countries, and its prevalence is increasing [1, 2]. The nasal mucosae and conjunctivae are mainly affected in response to normally innocuous airborne particles (allergens), being characterized by a complex of symptoms, including nasal pruritus, sneezing, rhinorrhea and nasal congestion [3, 4], as well as ocular irritation and tearing.

Many factors affect response to allergens in susceptible individuals, including the level of allergen exposure [5, 6]. Variable exposure to allergens in different geographical locations may contribute to the variation in prevalence of allergy internationally, or may provoke symptoms to different extents in sensitized individuals. Evidence of a positive association between atmospheric pollen counts and the prevalence of AR [7], however, has been disputed [6]. A Westernized lifestyle, including improved hygiene and the widespread use of vaccines and antibiotics, is thought to increase the likelihood of atopy [8], while climatic factors (e.g. temperature, humidity, wind currents and precipitation) are additional variables.

Although AR is not a life-threatening condition, it is associated with troublesome and at times debilitating symptoms, which can cause sleep loss, daytime somnolence, learning impairment, decreased cognitive function, decreased productivity and impaired quality of life (QoL) [9–12]. In addition, AR can be associated with comorbid
conditions, including asthma, sinusitis, otitis media, pharyngitis, laryngitis and conjunctivitis [13]. In 1995, AR cost the US economy $2.7 billion in direct and indirect costs [14, 15], and children lost approximately 2 million school days because of this condition [15]. It is, therefore, important that AR is managed actively and treated optimally to minimize the impact on QoL, comorbidities and costs.

**Mechanism of allergic response**

Individuals with a genetic predisposition for allergic reactions are described as being ‘atopic’. Atopy is defined by the European Academy of Allergology and Clinical Immunology (EAACI) Task Force as ‘a familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms, such as asthma, rhinoconjunctivitis or eczema/dermatitis’ [16]. Symptoms are triggered by immune hyper-responsiveness to otherwise innocuous environmental stimuli (allergens), which include pollens, house dust mites (HDMs), moulds, cockroaches and animal dander. Reactivity to several allergens is common and their considerable cross-reactivity [17, 18] makes it almost impossible to eliminate exposure completely because of their widespread distribution.

Development of an allergic response involves three key stages:

- **Sensitization**: generation of allergen-specific T helper 2 (Th2) cells.
- **Early-phase**: IgE cross-linking, mast-cell degranulation and release of allergic mediators.
- **Late-phase**: sustained response because of inflammatory cell recruitment, cytokine release from Th2 cells and perpetuation of IgE production.

In susceptible individuals, initial ‘sensitization’ is required for an allergic reaction to develop. The reaction is mediated predominantly by IgE, leading to mast-cell activation. The degree of sensitization required differs between individuals and varies according to the size, concentration and allergenicity of the antigen; for instance, pollen grains of less than 10 μm are more allergenic than larger grains [19]. Once inhaled, the allergen is phagocytosed by antigen-presenting cells, which process the resulting peptide fragments. The epitope of the antigen is presented to major histocompatibility protein class II molecules on T lymphocytes, which leads to the generation of antigen-specific Th2 cells and B cells.

The early phase of the allergic reaction is typified by elevated levels of IgE. IgE binds to its high-affinity receptor (FcεRI) on mast cells and other effector cells, which, in a sensitized individual, leads to cross-linking of IgE–FcεRI complexes. This results in mast-cell degranulation with the release of histamine, leukotrienes, chemokines and cytokines, such as interleukin (IL)-4, IL-5 and IL-6, tumour necrosis factor-α and granulocyte-macrophage colony-stimulating factor. A sustained inflammation follows, known as the late-phase response. This is maintained by Th2 cells and other inflammatory cells, such as eosinophils and basophils, which enhance IgE production.

**Clinical presentation of allergic rhinitis**

**Symptom patterns – severity and duration.** Mast-cell degranulation results in rhinorrhoea, sneezing, nasal obstruction and nasal and conjunctival itching – the classic symptoms of AR. Traditionally, AR has been subcategorized based on whether these symptoms are experienced for part of the year or all year round as follows:

- **Seasonal AR (SAR)**: triggered by pollens and/or moulds that are only present for certain months of the year.
- **Perennial AR (PAR)**: triggered by HDMs, indoor moulds, cockroaches or animal dander, all of which may be present year round.

In 2001, the World Health Organization (WHO) published guidelines on Allergic Rhinitis and its Impact on Asthma (ARIA), and proposed a new disease classification on which to base rhinitis treatment [20]. This model categorizes rhinitis according to persistence and severity of symptoms, with severity criteria based on the impact that symptoms have on QoL (Table 1).

**Priming.** The priming effect has been defined as an increase in reactivity of the nasal membrane following repeated exposure to pollen [21]. The application of this effect in an allergen challenge chamber (ACC) environment is to increase the level of sensitivity to a specific

<table>
<thead>
<tr>
<th>Classification</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent</td>
<td>≤ 4 days/week or ≤ 4 weeks/year</td>
</tr>
<tr>
<td>Persistent</td>
<td>&gt; 4 days/week and &gt; 4 weeks/year</td>
</tr>
<tr>
<td>Mild</td>
<td>All of the following: Normal sleep, No impairment of daily activities, Normal work, No troublesome symptoms</td>
</tr>
<tr>
<td>Moderate-to-severe</td>
<td>One or more of the following: Abnormal sleep, Impairment of daily activities, Abnormal work, Troublesome symptoms</td>
</tr>
</tbody>
</table>

Adapted with permission from the Chairman and Co-Chairman of ARIA.
allergen (e.g. ragweed or grass pollens), mimicking the natural process and facilitating the development of adequate symptoms for subject participation. A mucosal response to one allergen has been found to lead to increased sensitivity (or reduced symptom threshold) to other allergens, related to a variety of factors arising from earlier exposures [5, 22, 23]. Factors affecting the rate and degree of symptom development during the priming process are under study [24]. The Environmental Exposure Unit (EEU) in Kingston uses the priming effect by exposing subjects to ragweed pollen for one or more times in the 2 weeks prior to the study date.

Priming, both inside and outside of an ACC environment, usually develops early and may be ongoing, depending in part on current allergen exposure. For instance, it has been reported that at the beginning of the season, 90% of people with sensitization to Betula pollen develop symptoms with pollen counts higher than 80 grains/m³, but by the end of the season, counts that induce symptoms decrease to 30 grains/m³ [5]. Furthermore, this may be exacerbated by the presence of Alnus and Corylus pollens, with which Betula shares antigens [5]. This heightened sensitivity may also make an affected individual sensitive to other triggers or to irritants, such as tobacco smoke, which may not normally have caused a reaction [15].

Management of allergic rhinitis

Allergen avoidance. Most guidelines for AR suggest that allergen avoidance techniques should be an integral part of a management strategy [20]. It should be attempted in order to mitigate symptom severity [25]; but allergen avoidance has had limited success, in particular, because of widespread distribution of seasonal allergens [26, 27]. Therefore, management of AR usually includes therapeutic intervention.

Medications. Currently, there are five classes of drug treatments for AR (Table 2), each of which targets a different stage of the allergic reaction. These therapies may behave synergistically when used in combination, allowing better control of symptoms [28].

Histamine, released from the cytoplasmic granules of basophils and mast cells, plays a major role in AR. Antihistamines are currently the mainstay of AR treatment because of their effectiveness in controlling symptoms [14, 29]. They are competitive, reversible antagonists of histamine at H1-receptor sites on nasal mucosae, but they do not prevent histamine release or bind to histamine that has been released already. Antihistamines effectively alleviate symptoms attributed to the early-phase reaction, such as rhinorrhoea, pruritus and sneezing [29]. They also affect the late-phase reaction by reducing eosinophil infiltration and adhesion molecule expression [30].

Intranasal corticosteroids have a longer onset of action than antihistamines; although they are intended to be taken regularly, rather than as needed (PRN) [29], recent evidence of earlier onset indicates a possible PRN role in certain situations [31]. Intranasal corticosteroids, which have been found to be effective at controlling chronic inflammation are the first choice in the treatment of moderate-to-severe symptoms, and may be used concomitantly with oral antihistamines [20].

Cromolyns, administered intranasally, are sometimes used for mild, persistent disease, but are less effective than antihistamines or corticosteroids [29]. Leukotriene modifiers are indicated for the treatment of asthma and represent a useful approach to the treatment of AR, especially when used in combination with other treatments [32].

Owing to the range of treatments available, it is important that physicians have adequate information regarding the different properties of each drug so that they can prescribe the most appropriate agent for their patients. Medications should have a rapid onset of action, which is considered to be the most desirable property when selecting a treatment for rhinitis [33], as most

<table>
<thead>
<tr>
<th>Class</th>
<th>Example agents</th>
<th>Target</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihistamines</td>
<td>Desloratadine</td>
<td>H1 receptors</td>
<td>Antagonism of H1 receptor, preventing activation of H1 receptor-containing cells</td>
</tr>
<tr>
<td>Leukotriene modifiers</td>
<td>Montelukast</td>
<td>Leukotriene receptors</td>
<td>Competitive blockade of leukotriene receptors</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Beclomethasone</td>
<td>Chemokines</td>
<td>Inhibition of IL-1, TNF and prostaglandin synthesis</td>
</tr>
<tr>
<td>Cromolyns</td>
<td>Nedocromil</td>
<td>Mast cells</td>
<td>Inhibition of histamine release, mast-cell stabilization</td>
</tr>
<tr>
<td>IgE antibodies</td>
<td>Omalizumab</td>
<td>IgE</td>
<td>Prevention of IgE binding to mast cells</td>
</tr>
</tbody>
</table>

IgE, immunoglobulin E; IL, interleukin; TNF, tumour necrosis factor.
patients do not use medications regularly, even when they are prescribed for daily use [34].

An ideal AR treatment would:

- be effective in most subjects;
- provide fast symptom relief;
- prevent development of chronic inflammation or manage it if it begins;
- maintain activity over the full 24 h period between doses;
- require no co-medications;
- be effective against all individual rhinitis symptoms;
- have a good safety profile, conducive to long-term use;
- also be effective for non-rhinitis symptoms;
- improve QoL.

**Immunotherapy.** Allergen immunotherapy, in the form of repeated oral, sublingual, intranasal or subcutaneous administration of standardized allergen extracts, has been shown to be effective in reducing the intensity of symptoms and need for medications, as well as improving QoL [20, 35, 36]. A recent meta-analysis has confirmed the efficacy of sublingual immunotherapy (SLIT) [37], and double-blind, placebo-controlled trials have shown subcutaneous and SLIT to be comparable and significantly better than placebo for treatment of AR [38].

Subcutaneous injections carry the risk of local reactions at the injection site or more serious systemic reactions, such as anaphylaxis [39]. Postmarketing surveillance studies, however, have indicated a more favourable safety profile for SLIT [20], even in paediatric populations [40, 41]. Studies have indicated that SLIT can modify the course of the disease [35], restricting development of asthma in subjects with AR [42, 43]. However, the long-term efficacy of SLIT needs to be evaluated further. As such, immunotherapy should not be considered an ultimate treatment of respiratory allergy, but a therapeutic tool to be harmonized with pharmacological treatments [20].

**Allergy research techniques**

Traditional SAR trials are performed during the pollen season for up to several weeks. However, a number of variables, including differences in pollen levels over the course of the study, subject compliance and recall bias, reduce their sensitivity. When trials are conducted in multiple locations, some of these variations (such as pollen levels) may be exaggerated and are harder to adjust for. In addition, because study participants are not monitored, traditional trials are not suitable for the determination of consistent, frequent, precisely timed symptom assessments [44]. These factors contribute to a traditional trial’s insensitivity for detecting onset and duration of action differences between treatments, or differences between various doses of the same treatment. This may be one explanation as to why onset of action is an endpoint that would be difficult to monitor in traditional studies and why it is rarely measured.

The essential factor in establishing drug efficacy in AR is the ability to differentiate placebo from treatment effect. Of importance, but difficult to establish, are clinically meaningful differences between active treatment arms. In order to prescribe the most effective agent for each patient, physicians need to be able to characterize important differences between treatments.

Randomized, controlled trials (RCTs) are accepted as the standard method of assessment of treatment efficacy and safety, and a well-designed RCT for AR interventions should:

- control allergenic stimuli and alleviate variations in exposure;
- select subjects sensitive to a common allergen, with a spectrum of response;
- rigorously evaluate symptoms in response to treatment;
- search for and identify adverse events;
- be clinically relevant to ‘real life’.

**Assessing clinical outcomes**

**Subjective evaluation.** Subject self-reporting of response is central to the investigation of AR treatments. Trial participants are asked to evaluate the frequency and severity of their symptoms, using, for example, a verbal scale (Table 3), visual analogue scale (VAS) [45] or graphical scale.

Although symptom scores are subjective, they provide a measure of the effect of an allergen in an individual and give the investigator the ability to look at change in individual symptoms over time. In addition to nasal symptoms, ocular symptoms of AR can be assessed by the extent of conjunctival itching, redness and tearing. Individual symptoms can also be grouped to give an overall score, for example the major symptom complex (MSC) or the total symptom complex (TSC) scores (Table 4) [46, 47].

**Objective evaluation.** Objective evaluation of respiratory parameters supports subjects’ subjective rating of symptom severity and treatment response. Techniques include rhinomanometry, weighing of nasal secretions in paper

| Table 3. Example of a 4-point verbal scale for measurement of allergic rhinitis symptoms |
|-----------------------------------|---------------------------------|
| Score   | Symptoms                                      |
| 0 = None | No symptom(s) evident                           |
| 1 = Mild | Symptom(s) present but easily tolerated          |
| 2 = Moderate | Definite awareness of symptom(s); bothersome but tolerable |
| 3 = Severe | Symptom(s) hard to tolerate; interferes with daily activities |

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tissues, nasal peak inspiratory and expiratory flow, nasal endoscopy, nasal lavage and spirometry (Table 5). More than one measurement may be used: for example, the sneeze count and the weight of nasal secretions can be combined as a ‘sneeze–drip’ score. Beyond respiratory assessments, digital imaging can be used to evaluate the conjunctival vascular reaction [51], and serum levels of proinflammatory mediators can also be monitored.

Quality of life and global evaluation. The impact of AR on QoL is considerable, and it is important to include this in the medication assessment [52]. QoL questionnaires can be used, and accepted questionnaires include the Medical Outcomes Study 36-Item Short Form (SF-36) Health Status Questionnaire [54] or the Rhinitis Quality of Life Questionnaire (RQLQ) [55]. Use of these surveys allows the effects of rhinitis on QoL to be acknowledged, which may otherwise be ignored or trivialized [15].

### Table 4. Example of two multisymptom scores for assessment of allergic rhinitis [46, 47]

<table>
<thead>
<tr>
<th>MSC score</th>
<th>TSC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runny nose</td>
<td>MSC</td>
</tr>
<tr>
<td>Sniffles</td>
<td>Itchy eyes and ears</td>
</tr>
<tr>
<td>Itchy nose</td>
<td>Itchy throat</td>
</tr>
<tr>
<td>Watery eyes</td>
<td>Cough</td>
</tr>
<tr>
<td>Sneezes</td>
<td>Postnasal drip</td>
</tr>
<tr>
<td>Nose blows</td>
<td></td>
</tr>
</tbody>
</table>

*Symptoms rated on a 5-point scale, except for sneezes and nose blows (8-point scale). MSC, major symptom complex; TSC, total symptom complex.

### Table 5. Techniques for obtaining objective data on allergic rhinitis

<table>
<thead>
<tr>
<th>Technique</th>
<th>Data obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weal and flare skin challenge</td>
<td>Although not a true respiratory parameter, this allows pharmacological data between drugs to be assessed and compared using the skin response to applied histamine and/or allergen</td>
</tr>
<tr>
<td>Rhinomanometry</td>
<td>Sensitive assessment of nasal obstruction (nasal airflow, resistance and airflow increase) by measuring the difference between prenasal and postnasal pressure and the corresponding nasal flow</td>
</tr>
<tr>
<td>Acoustic rhinometry</td>
<td>Audible sounds (150–10,000 Hz) that are propagated in a tube are affected by nasal impedance, helping to define structural and mucosal components of the nasal passage</td>
</tr>
<tr>
<td>Rhinostereometry</td>
<td>Nasal mucosae are observed through a surgical microscope to detect changes in mucosal swelling</td>
</tr>
<tr>
<td>Nasal endoscopy</td>
<td>Provides an endoscopic image of the nasal cavity, which can be recorded as digital images or video for comparison with subsequent investigations. To calculate nasal patency and severity of turbinate swelling, the images are rated according to a 5-point scale (0 = nasal airways completely open; 4 = nasal airways completely obstructed). The sum of scores from both nostrils indicates the clinical significance of symptoms</td>
</tr>
<tr>
<td>Nasal peak inspiratory and expiratory flow</td>
<td>Simple method of assessing nasal obstruction, the maximal inspiratory and expiratory nasal airflow volumes being expressed in L/min</td>
</tr>
<tr>
<td>Nasal secretion</td>
<td>Nasal secretions can be collected in a sealed container or on tissues, either of which are weighed before and after use to estimate the severity of nasal secretion</td>
</tr>
<tr>
<td>Nasal lavage</td>
<td>Nasal micro-lavage can be performed during and after allergen exposure. Allergic mediators such as histamine, prostaglandins and leukotrienes can then be quantified [48–50]. Nasal levels of ECP can also be measured</td>
</tr>
<tr>
<td>Spirometry</td>
<td>Forced expiratory volume in 1 s (FEV1) is monitored at baseline and during allergen exposure</td>
</tr>
<tr>
<td>Conjunctival digital imaging</td>
<td>Selected areas (2.3–3.5 mm) of the conjunctivae are documented by digital imaging during allergen challenge sessions to assess changes in conjunctival vascular reaction [51]</td>
</tr>
<tr>
<td>Ocular hyperaemia and oedema</td>
<td>Examined using a slit lamp to support conjunctival digital imaging results</td>
</tr>
<tr>
<td>Serum ECP</td>
<td>Serum levels of ECP are higher in subjects with an inflammatory allergic response than in non-atopic individuals</td>
</tr>
<tr>
<td>Exhaled and nasal nitric oxide</td>
<td>Levels of exhaled and nasal nitric oxide are elevated in individuals with allergic bronchial and nasal inflammation</td>
</tr>
</tbody>
</table>

ECP, eosinophil cationic protein.

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Skin prick tests. Skin prick challenge is a simple method, with both allergen and histamine being easy to apply. Skin provocation with histamine is one of the methods used most commonly for preliminary evaluation of medications for AR [57]. It is also a cheap, rapid and accurate method of identifying allergen sensitivity, whereby mast-cell activation in the skin causes a characteristic ‘weal and flare’ reaction in response to allergen administration. The skin prick test is often a precursor to nasal allergen provocation tests. Data show a correlation between skin and upper respiratory effects of antihistamines [58], and there is a long-standing tradition of extrapolating data from the skin to the airways in clinical practice. However, the use of data from these studies may not be predictive of the clinical efficacy of antihistamines for rhinitis symptoms and has been challenged [59].

Ocular provocation test. The standard ocular (or conjunctival) provocation test begins with a titration of the selected allergen applied to both eyes of each subject. This enables gradation of symptoms using standardized scales. The quantity of allergen applied is increased up to a predetermined threshold of response, which enables a difference to be seen between drug and placebo responses. In subsequent sessions, placebo solution is applied to one eye and the study drug to the other, before challenge with allergen in both eyes. The eyes can then be monitored for symptoms of allergy, and the responses compared [51, 60].

This method enables determination of the onset and duration of action of treatments. By enrolling subjects based on their initial response to allergen, only individuals with sufficient responses are included, and the titration provides a mechanism to ensure sufficient reactivity. One advantage of this method for evaluating the pharmacodynamics of a treatment is that the other eye can serve as an easily accessible control [61]. However, as the allergen concentration applied is usually higher than environmental levels, this must be accounted for when determining clinical relevance. Furthermore, information obtained from an ocular challenge cannot be extrapolated directly to the respiratory system. Therefore, in AR research, this method offers supporting data only.

Nasal provocation test. Nasal provocation tests (NPTs) to histamine or suspected allergens provide meaningful results, and a correlation between skin tests and NPTs has been reported [62]. NPTs are now recognized by the international Global Resources In Allergy (GLORIA™) [63] as an additional resource for the diagnosis of AR.

There are a number of techniques for the application of allergen to the nasal mucosa, including delivery of allergen in powdered form, via sprays or nebulizers, from a syringe, topically using cotton wool or by impregnation onto paper discs. Response to nasal provocation can be assessed both subjectively and objectively by a variety of methods, as listed in Table 5. An advantage of the nasal provocation test is the potential for critical evaluation of the kinetic response to stimuli, rechallenge and treatment [23].

Traditional outpatient studies. Traditional outpatient trials for AR are randomized and double blinded to compare the drug of interest with placebo and one or more active agents over a period of usually 2 or more weeks while study participants carry on with their normal daily activities. SAR trials are undertaken during the pollen season and are therefore restricted in timing and duration. PAR trials are conducted out of the allergy season to avoid the confounding effects of pollen exposure. Subjects are given diaries to complete at home for the duration of the study to record symptoms of rhinitis and satisfaction with treatment over 12 or 24 h intervals. This reflective scoring provides an estimate of the overall effectiveness of the treatment over the observed time period. In addition, an end-of-dosing-interval score can be recorded to evaluate the duration of effect. Diurnal variations, commonly seen in subjects with AR, can also be monitored.

Parallel-group trials compare placebo with one or more active treatment arms, with each randomized group receiving a different study treatment. These are usually multicentre studies, as they require large numbers of subjects per group to achieve sufficient statistical power to see even small differences between the study arms. A crossover design may be used in PAR trials where a single group of subjects successively receives each of the study medications, and each treatment course is separated by a wash-out period. This methodology requires fewer subjects than the parallel-group design, but assumes that AR is stable within one individual over time, and that the selected washout period eliminates the carry-over effect between treatments. Crossover designs are logistically difficult to carry out in traditional SAR trials, mainly because of the short duration of the pollen season in many places [64] and the priming effect [21].

The traditional outpatient trial is the most commonly used method for the investigation of medications for AR. It is the best characterized approach and is recognized as such by regulatory authorities, who provide guidelines on methodology and interpretation of results of clinical trials. It is the only study system to date that is fully accepted as representing a ‘real life’ setting.

However, there are a number of limitations to traditional outpatient trials. Although widely considered to reflect ‘real life’, they rely on a number of assumptions and are unable to account for the high number of uncontrolled variables, making them relatively insensitive for the determination of subtle differences between treatment arms. Notably, there are large variations in the timing of the pollen seasons and in diurnal and annual pollen counts.
Growth conditions, altitude [65] and meteorological factors such as wind or rainfall continually influence concentrations of pollen in the atmosphere [66, 67], producing considerable variation in and between seasons [68]. However, regional pollen counts do not necessarily represent the levels experienced by any one individual, as work and recreational factors contribute to the level of personal pollen exposure. Thus, wide differences in subject exposure occur, giving variable baseline symptom levels that are hard to correct for in subsequent analyses. Additionally, variations in pollen levels from location to location probably contribute to differences in results obtained at different sites in multicentre studies. These factors, as well as variations in pollen levels from year to year, make it almost impossible to reproduce trial conditions and results. A significant placebo effect is often observed in such study settings, which may be because pollen exposure is insufficient to induce symptoms throughout the study, misleadingly implying a protective or treatment effect, especially later in the trials when interpretation of medication effect would coincide with the normal decline in seasonal pollen levels [69].

In addition to the variability of pollen exposure, subject compliance with study procedures such as the acknowledged last-minute completion or backdating of symptom assessments also contributes to reduced sensitivity in the data from traditional trials. It is also difficult to accurately assess compliance with trial medications, and whether other medications or interventions that might affect response to the study medication have been taken. The dropout rate in outpatient trials is often high, meaning that a number of study centres are needed to ensure sufficient subject numbers to obtain statistical power. Thus, despite following the same protocol, the heterogeneous population and study conditions used increase the likelihood of a spread of results, often leading to significant differences in the results from different trial centres.

Investigators, in response to the variables associated with traditional clinical trials, have developed alternative strategies such as day-in-the-park studies and ACCs to limit some of these variables and to better define performance of anti-allergic medications [44].

**Day-in-the-park studies.** When outpatient trials are conducted at multiple centres and subjects follow their usual lifestyle for the duration of the study, there is a wide variation in the frequency, duration and intensity of allergen exposure among the participants. Day-in-the-park studies, first conducted in the late 1970s [70], attempt to limit this variation in exposure by conducting SAR studies outdoors, over 1–2 days. By selecting a park location, the objective is to assure pollen exposure and limit other variables. Numerous park studies have been published [71–78].

Trials are conducted during the pollen season – ideally when allergen levels are at a peak – and are randomized, double-blind, parallel-group designs. A large number of potential subjects are screened for sensitivity to the prevalent pollen and other inclusion/exclusion criteria, and qualifying subjects are asked to attend the treatment day at a prearranged site. Subjects are exposed to ambient allergens in the park setting and asked to rate their symptoms on diary cards. If they attain symptoms of a sufficient level, they are given a medication and remain at the trial site, under observation by the investigators [72]. This ensures that subjects are exposed to similar environmental conditions, receive treatment at the same time and report symptoms regularly, thus avoiding missing data. Subject activities, including physical activity and food intake (e.g. citrus), are also monitored, and variables such as medication compliance are recorded. Subject compliance with procedures, including completion of diary cards, is easier to control and, because of the short duration of trials, continuous attendance of subjects is improved and monitoring by staff throughout the trial is possible.

This method provides pharmacodynamic data such as onset and duration of action, but because of the usual short-term nature of these studies, there may be limited information on safety. However, there are practical difficulties in setting up day-in-the-park trials, as the pollen season is limited and there is the risk of disruption by adverse weather conditions. Furthermore, the peak of the pollen season can only be estimated and thus maximal allergen exposure is not assured [44]. Intra-individual allergen exposure is difficult to determine and is not standardized during the trial period. Pollen levels are not always recorded and publications often lack supporting evidence as to their relationship with the seasonal peaks in pollen levels [44]. Variable pollen levels within and between seasons remain confounding variables that make repetition of results difficult.

**Allergen challenge chamber studies.** An ACC is a specially designed room that hosts study participants in a controlled environment, in which AR symptoms, treatment effect and differences between treatments are assessed in response to ambient allergen. The development of ACCs began in the 1980s [79], with the first clinical trial published in 1988 [80]. The technique is based on the concept that uniform exposure to controlled levels of allergen enables an accurate analysis of treatment efficacy without the variables inherent in traditional studies. The ACC has been recognized as being at least equivalent to histamine or allergen provocation models, with established reproducibility of results [81–83].

The air within an ACC is filtered to remove extraneous allergens and pollutants (Fig. 1), and is strictly controlled and adjusted for allergen type and concentration,
temperature, humidity, CO₂ concentration and airflow. Allergen concentration is the most important constant in this system – a well-documented, uniform concentration of allergen must be achieved throughout the exposure area of the chamber, and the concentration is carefully selected to be relevant to levels found outdoors. The aim is for all subjects to be exposed to the same predetermined concentration of allergen, regardless of their location within the chamber or the time of their participation, as measured by regular sampling during the study period. The resulting conditions enable highly reproducible results that are consistent throughout each study.

Conventional nasal or conjunctival challenge tests may induce a response in normally asymptomatic subjects because of the tendency to administer high allergen concentrations in the absence of a predefined ‘maximum dose’ [84]. Levels of allergen in an ACC (i.e. peak seasonal) induce responses in only those subjects who would generate a response in ‘real life’ [85]. Furthermore, non-allergic staff members do not report symptoms, despite repeated pollen exposures over a number of years in ACC settings.

Studies conducted in ACCs to date have been monocentric, with the lead investigator ensuring consistency of conditions in each session throughout the study [46, 47, 86, 87]. Parallel-group or crossover designs can be used, with single or multiple medication intakes and with single or multiple allergen types. SAR studies may be conducted both in and out of the pollen season in an ACC. In-season studies allow control of many factors that cannot be regulated in traditional trial methodologies. Additionally, there is less priming required compared with out-of-season studies, especially for subjects who are already symptomatic (primed). The capability of conducting studies year round also offers scheduling advantages independent of pollen exposure conditions.

In the EEU, subjects are exposed to a predetermined concentration of allergen during the priming phase. Participants who attain sufficient allergic symptoms can then be randomized to participate in the treatment phase, where they are exposed to the single test allergen at concentrations at which they have demonstrated sensitivity during priming. As other parameters are controlled, differences in response observed between treatment groups should be because of differences in drug activity.

Both subjective and objective data are retrieved, with data being immediately available. Participants take medications at a precise time(s) and complete symptom diaries at specified intervals under constant observation by study personnel. Thus, timely symptom assessments and medication compliance can be ensured. This helps to avoid the typical problems in outpatient trials of non-compliance, missing data or inaccurate ‘last-minute’ diary completion, and makes the ACC especially suitable for assessing precisely timed efficacy measures such as onset of action.

ACCs can overcome many of the problems associated with traditional trials. As an ACC permits large amounts of precise numerical data to be gathered, statistically significant results can be obtained with far fewer subjects than would be needed in an outpatient trial. This also has ethical considerations, as fewer subjects are exposed to investigational agents or placebo. Subjective data can be supplemented with periodic objective measurements, such as nasal inspiratory flow rate [31], lung function [88] or conjunctival vascular reaction [51]. Additionally, nasal secretions can be used to measure the release of allergic mediators and cytokines at any point [50].

Challenge chambers permit assessment of the relative efficacy of anti-allergic medications and also allow the possibility of exploring the dynamics of allergic symptoms and their impact on cognitive function. ACCs provide a uniquely cost-effective setting for bioequivalence studies of both topical nasal products and eye preparations, and they are an ideal method for proof-of-concept studies for agents in early Phase II development. At this stage of drug development, the results obtained in animal studies can be confirmed or rejected in humans, and ACC studies can establish this within a short timeframe, with evident advantages.

ACCs allow precisely timed measures of efficacy at regular intervals, and data on onset and duration of action can be obtained more accurately than with traditional trials. The rapid alleviation of symptoms is one of the most important factors for a subject, ensuring treatment compliance, satisfaction and overall QoL. In recognition of this, onset and duration of action have been recommended by the ARIA/EAACI workshop group as important pharmacodynamic characteristics of anti-allergic medications, to facilitate once-daily administration of treatments and clinical efficacy at the end of each dosing period [89].

QoL has been evaluated in ACC trials, both to examine the impact of studies where subjects’ allergic symptoms are induced as part of the study design [90] and to determine if there is a relationship between QoL score and the likelihood that a study participant would respond to placebo [53]. A QoL questionnaire expressly designed for ACC trials is in the process of being validated. ‘Real
life’ pathophysiological mechanisms such as diurnal variability, or long-term endpoints such as pharmacoeconomics, have not yet been explored.

**Guidelines on conducting allergic rhinitis trials**

**Difficulties in conducting rhinitis trials**

The Food and Drug Administration (FDA) (FDA guidance remains draft and not for implementation) and European Medicines Agency (EMEA) have both issued guidance/guidelines on the clinical development of medications for the treatment of AR, and both acknowledge the difficulties that exist in conducting meaningful trials on this subject [3, 4]. Key issues include the following:

- the likelihood that subjects will have multiple allergic triggers or comorbidities with overlapping symptoms, such as sinusitis, allergic conjunctivitis or asthma [3, 4];
- recruitment of subjects who turn out to be asymptomatic or have only mild rhinitis symptoms at baseline [3, 4];
- the subjective nature of subjects’ symptom assessments and self-reporting of compliance [3];
- inter- and intra-individual variability in allergic exposure during the study [3, 4];
- the difficulty in estimating the peak of the pollen season [3];
- spontaneous variability of severity and nature of symptoms within an individual [4];
- the wide variety of study designs, with different endpoints, durations and data analysis techniques [4];
- the need to consider multiplicity (multiple possible rhinitis symptoms, numerous repeated measures in individuals) and a possible need to make post hoc changes to the protocol or analysis (e.g. redefinition of primary efficacy, change in the analysis set or redefinition of the pollen season) [4].

**Food and Drug Administration and European Medicines Agency recommendations for research**

Both the FDA guidance and EMEA guidelines note that studies during the developmental programmes for a new anti-rhinitis drug often fail to demonstrate the effectiveness of the treatment [3, 4]. Each recommends at least two adequate and well-controlled Phase III trials for approval of a SAR or PAR indication for a new product [3, 4]. The dose–response relationship must be evaluated using either clinical (traditional outpatient trials) or validated pharmacodynamic studies [4]. Randomization, placebo control and an active control arm are all required to account for the variable nature of the disease and the subjectivity of data collected [3, 4]. Studies should be double-blind, parallel-group designs, ideally with a placebo run-in period [3]. Non-inferiority trials are not possible because of lack of sensitivity, and superiority trials should be conducted against a well-established comparator with the same route of administration [4]. Non-inferiority cannot be claimed from superiority trials in the absence of a placebo arm for internal validation [4].

Pollen counts should be measured at the different study centres, to document the exposure of subjects to relevant allergens during the study period [3, 4]. However, personal exposure is dependent not only on weather but also on individual factors such as frequency and duration of outdoor exposure. Personal monitoring of exposure, although possible, is technically complicated and not feasible in large-scale trials [64]. Randomization of subjects in each centre should be conducted over a short time period to reduce variability in allergen exposure, and PAR trials should be conducted out of the pollen season [3]. The FDA guidance states that the duration of a double-blind period should be at least 2 weeks for SAR and 4 weeks for PAR [3]. The EMEA guidelines note that the study duration may vary depending on the onset of action of the product, indication sought (treatment vs. prevention of symptoms) and duration of allergen exposure expected [4]. In traditional clinical studies, the EMEA-recommended duration for the double-blind period of SAR trials is 2–4 weeks, and 6–12 weeks for PAR trials [4].

Safety is of the utmost importance for AR treatments – products are likely to be used over the long term, and although rhinitis may be chronic and disabling, it is not life threatening. Thus, safety data from long-term exposure for up to 12 months in at least 100 subjects [91] should be available. Subjects with comorbid conditions may be included in trials for the purposes of safety analyses only [4].

Subjects with asthma require special consideration, as asthma and AR commonly occur together. Subjects with mild intermittent asthma are included routinely in trials, although those with at least moderate asthma triggered by the study allergen are usually excluded to avoid exacerbation of symptoms. The FDA states that subjects with asthma, with the exception of mild, intermittent asthma, should be excluded [3], while the EMEA allows inclusion of asthmatic subjects for the purpose of obtaining safety data [4].

Even though regulatory authorities acknowledge the role of the ACC setting, referred to as an environmental exposure unit in their guidelines/guidance, the EMEA requires that justification is provided for its use and validity, and both the FDA and the EMEA restrict this to a limited role and provide no guidance on how to assess the results [3, 4]. Neither is any guidance given for how to interpret day-in-the-park studies. Formalization and standardization of trial methodology and analysis would allow more homogeneous data sets to be collected, enabling better comparisons between different trial types.
Allergen challenge chambers: Food and Drug Administration guidance

Three settings are recommended by FDA guidance to study the onset of action of agents, one of which is the ACC [3]. Results from the ACC, as well as those derived in the park setting, must be replicated if they are used to support an onset of action claim that is shorter than that observed in Phase III trials (i.e., a traditional outpatient study), despite both models being acknowledged as settings for assessing onset of action [3]. This is because of the shorter duration of these studies, the restricted setting and the manner in which they are conducted. The use of an ACC in SAR prophylaxis trials is also recommended [3].

Allergen challenge chambers: European Medicines Agency guidelines

The ACC model is included within current EMEA rhinoconjunctivitis drug development guidelines as a possible pharmacodynamic assessment tool, to provide supportive evidence of superiority over placebo and to compare local vs. systemic therapy for AR and conjunctivitis [4]. The EMEA also lists validated pharmacodynamic studies as an alternative to clinical studies for establishing the effective dose range and optimal dose of a treatment, provided the route of allergen challenge is made in line with the intended indication. However, the ACC is currently grouped in the same investigative category as the nasal allergen challenge and conjunctival allergen challenge, and is not yet recognized by the EMEA as a valid, stand-alone method for therapeutic efficacy.

Allergen challenge chambers

Physical set-up

The number of ACCs available worldwide is limited because of the complexity of the physical set-up required to ensure a controlled density of particles. However, several ACCs exist, with more under development. In the order of longest standing, the chambers commonly used in allergy RCTs are:

- the Vienna Challenge Chamber (VCC) at the University Clinic, General Hospital, Vienna, Austria;
- the VCC at the Allergy Centre, Vienna West, Austria;
- the EEU at Kingston General Hospital, Ontario, Canada;
- the Chamber at National University Hospital, Copenhagen, Denmark;
- the Allergen Exposure Unit (AEU) in Atlanta, Georgia, USA;
- the Environmental Exposure Chamber (EEC) at Allied Research International, Mississauga, Ontario, Canada;
- the ACC at Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany;
- the EEU Wakayama, Wakayama, Japan;
- the ACC at Osaka Medical University, Osaka, Japan. (Although the EEC, the EEU Wakayama and the Osaka ACC have been identified as allergen challenge chambers, studies utilizing these units have not been published in peer-reviewed journals to date.)

The individual chambers possess some physical and technical differences (Table 6), but the characteristics are the same:

- studies are not limited to the period of natural pollination;
- controlled and uniform allergen exposure;
- no impact of weather conditions;
- no impact of personal context (participation in outdoor activities, etc.);
- ensured compliance (medication administration, timeliness and completion of symptom assessments);
- instantaneous and precisely timed symptom assessments.

As experience with ACC studies has been acquired mostly from the VCC (Vienna, Austria) and the EEU (Kingston, Canada), these allergen challenge systems will comprise the focus of discussion in subsequent sections.

Vienna Challenge Chamber, University Clinic of Vienna. The longest-standing allergen challenge system, the VCC, first discussed in the literature in 1987 [79], is situated in the University Clinic of Vienna, Austria. It was developed in 1985 and extensively rebuilt in the new building of the University Hospital in 1992. It is an enclosed system where up to 14 participants enter through an airlock and are challenged simultaneously, usually for between 2 and 8 h. The key features of the VCC are outlined in Table 7 and Fig. 2.

Environmental Exposure Unit, Kingston General Hospital. The EEU was first developed in 1981 as a system for testing the respiratory effects of urea formaldehyde foam insulation [96]. In 1987 it was permanently modified for allergen challenge. The system circulates fresh, filtered outdoor air and room air exits through ceiling vents. Pollen is dispersed into the airflow and propelled around the room by fans. Frequent sampling throughout the seating area gives an accurate measurement of allergen concentration during the study, and allergen levels are replenished to maintain a constant level during the challenge session [92].

The key features and physical set-up of the EEU are shown in Table 8 and Figs 3 and 4.

A controlled environment

The ACC has proved effective in multiple studies evaluating various aspects of AR. It has been used to investigate the priming effect of ragweed pollen, the onset of allergic
Table 6. Published, peer-reviewed accounts of the physical features and capacity of existing allergen challenge chambers

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td>Area: 13.78 m²</td>
<td>Area: 303 m²</td>
<td>Area: 21 m²</td>
<td>Area: 5 m²</td>
<td>Area: 360 m²</td>
<td>Area: 47 m²</td>
</tr>
<tr>
<td></td>
<td>Height: 2.7 m</td>
<td>Height: 2.74 m</td>
<td>Height: 2.8 m</td>
<td>Maximum height: 2.6 m</td>
<td>Height: 3.6 m</td>
<td>Height: 2.8 m</td>
</tr>
<tr>
<td><strong>Capacity (number of subjects)</strong></td>
<td>Specially constructed room, hosting up to 14</td>
<td>Modified room in a hospital setting, seating up to 160</td>
<td>Specially adapted room, hosting up to 24</td>
<td>Portable tent designed to be adaptable to an existing room, hosting a single occupant</td>
<td>Specially constructed room, seating up to 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Specially adapted room, seating up to 18 (up to 25 in subsequent studies)</td>
</tr>
<tr>
<td><strong>Ventilation/dispersion system</strong></td>
<td>Air enters through openings in the ceiling, and used air is sucked out through openings at floor level Second air circuit for allergen-loaded air delivered from the ceiling</td>
<td>100% outdoor air, filtered through Farr 30/30 and Farr Riga-Flo 200 filters Airflow of 3200 cfm Pollen feeder emits pollen into the unit</td>
<td>Filtered air, completely conditioned Second air circuit for allergen-loaded air</td>
<td>Inlet vent and micropore filter Allergen administered as aerosol through nebulizer connected to inlet pipe</td>
<td>Outdoor air ventilation at rate of ≥5 cfm/person HVAC supply airflow rate of ~1.67 cfm/square foot</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Filtered air (HEPA) with push-pull ventilation system</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Air is mixed by 14 swirl inlets for incoming air and thermal convection from floor heating</td>
</tr>
<tr>
<td><strong>Typical allergen load</strong></td>
<td>1500 grass pollen grains/m³ (700–2000 grains/m³)</td>
<td>350 ± 500 ragweed pollen grains/m³</td>
<td>1400 grass pollen grains/m³ (90 ng/m³ Der p 1)</td>
<td>Theoretical maximum of 50–100 ng/m³ Der p 1</td>
<td>3000–4500 ragweed pollen grains/m³</td>
<td>1000–8000 grass pollen grains/m³</td>
</tr>
<tr>
<td><strong>Allergen load monitoring</strong></td>
<td>Modified Burkard pollen traps make measurements every 5 min</td>
<td>Pollen counts collected by seven Rotorod samplers, every 30 min</td>
<td>Every 5 min</td>
<td>Portable air sampling unit Outlet air sampler</td>
<td>Pollen counts collected by five Rotorod samplers, every 30 min</td>
<td>Pollen concentration monitored every minute by a laser particle counter and every 30 min by two Rotorod samplers</td>
</tr>
</tbody>
</table>

cfm, ft³/min; Der p 1, Dermatophagoides pteronyssinus; EEU, environmental exposure unit; HEPA, high efficiency particulate air; HVAC, heating-ventilation air-conditioning; VCC, Vienna challenge chamber.
symptoms and the efficacy and safety of experimental drugs. The effectiveness of oral antihistamines, nasal corticosteroids, immunotherapy and ocular compounds are just some of the investigations conducted in ACCs and published in peer-reviewed journals to date (Table 9).

As would be expected, the types of symptoms assessed, the questionnaires used for data recording, the duration of a challenge session, the allergen concentration selected and other parameters vary between studies; each study and each chamber may have a different protocol.

**Homogeneous allergen distribution.** ACC studies typically involve exposure to a single allergen and subjects are exposed to the specified allergen during multiple sessions in an ACC. Inter- and intra-session allergen concentrations must be equivalent throughout multiple sessions within a study. In the VCC, for study protocols using different allergens, separate supply systems are installed for each allergen type in order to avoid contamination. Dispensers supply the chamber with allergen-loaded air at a constant rate, and a regulated, slightly turbulent airflow ensures homogeneous dispersion of allergen in the air. A feedback system prompts dispensers to add or recirculate allergens to maintain a constant level within the chamber.

The pollen load in an ACC is carefully controlled and its homogeneity is tested by frequent sampling throughout the room. For example, Fig. 5 shows the distribution of pollen in the VCC, which is within a narrow range in all areas of the chamber. Once subjects are seated in the chamber, the pollen distribution system is switched on (at \( t = 0 \) h). The output of the pollen distribution system is then adjusted based on subsequent sampling to achieve a steady state, which ensures homogeneous dispersion of allergen throughout the chamber. This has been demonstrated in the ACC in Hannover, where extensive

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### Table 7. Key features of the University Clinic Vienna Challenge Chamber (VCC)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
</thead>
</table>
| Chamber                  | Length: 5.25 m; width: 2.60 m; height: 2.70 m  
Area: 13.78 m²  
Volume: 37.20 m³  
Materials: Smooth aluminium surfaces to reduce allergen adherence (also antistatic measures and thorough cleaning between challenge sessions) |
| Airlock                  | Area: 1.30 m²                                                                                                                                 |
| Working space            | Area: 42.50 m²                                                                                                                                 |
| Number of participants   | Up to 14                                                                                                                                       |
| Ventilation system       | Indoor air ventilation system, blowing fresh air into the chamber through openings in the ceiling, and sucking out used air through six openings close to the floor  
Amount of fresh air calibrated with number of study participants |
| Temperature              | Usually set at 24–26 °C, fluctuating only within 0.5 °C  
Stable temperature reached 15 min after the start of the challenge session  
Recorded every 5 s at four locations around the chamber at a height of 2.2 m |
| Humidity                 | Usually set at 40–45%, fluctuating only within 1.5%  
Kept within 0.1% during challenge session, regardless of number of subjects and duration of session  
Recorded every 5 s at a single location, 0.5 m above the floor |
| CO₂ concentration        | 60 Pa lower in chamber than on the outside, to prevent contamination of the working area or other parts of the building  
Second air circuit for allergen-loaded air, with separated supplying system for different allergens to avoid cross-contamination  
Delivered by vacuum pressure from two locations on the ceiling  
Slow and continuous sedimentation of allergens occurs (e.g. 1 m/min for grass pollen) |
| Allergen dispersion system | Target concentration is generally:  
pollen: 1500 grains/m³ (700–2000 grains/m³)  
dust mites: 20–110 ng Der p 1/m³  
Monitored using:  
modified Burkard pollen traps – simultaneous volumetric measurements (allergen particles/m³) made at three levels and at nine different spots in each level, every 5 min. Thermo-anemometer automatically monitors the flow of the trap; slides automatically analyzed by light microscope  
cyclone samplers – accumulate and measure content of major allergen (ng). Immunohistochemical analysis using an enzyme-linked immunosorbent assay (ELISA) is performed  
Laser nephelometry – continual monitoring of the number of particles of certain diameters in the air |

**Der p 1, Dermatophagoides pteronyssinus.**
validation measurements have demonstrated homogeneous spatial pollen distribution within a range of ± 10% [88].

**Allergen types.** For the investigation of SAR, allergens used commonly include pollens from grass (e.g. *Phleum pratense* or *Dactylis glomerata*), birch tree (*Betula pendula*), ragweed (*Ambrosia elatior* or *Ambrosia artemisiifolia*) and Japanese red cedar tree (*Cryptomeria japonica*). The allergen to which most subjects with rhinitis are sensitive will vary between areas, with grass and birch pollens being key triggers in Europe [126], compared with ragweed, grass and tree pollens (birch, maple and oak) in North America, and Japanese red cedar pollen in Japan. The size, concentration and allergenicity (antigenicity) of pollens all play a role in the sensitization of subjects, and so pollen species and concentration are controlled for in an ACC study. Commercial ragweed pollens are available and have been shown to have the same allergenicity as fresh pollen. The same batch of pollen, which is characterized antigenically (antigen E), is used throughout a given study in the EEU in Kingston. The FDA also provides a reference pollen extract, enabling determination of pollen allergenicity – the Bioequivalent Allergy Unit. For PAR,
HDM (Dermatophagoides pteronyssinus) Der p 1 is used commonly.

**Allergen concentration.** In SAR trials, the aim of the ACC is to provide controlled allergen delivery at levels consistent with peak outdoor exposures to elicit the full range of allergic symptoms (from mild to severe) in allergic subjects without producing symptoms in non-allergic subjects [92]. Sensitivity varies among individuals, but the intensity of symptoms in response to grass pollen, for example, appears to be concentration dependent [88]. In real life, subjects may be exposed to a wide range of allergen concentrations of up to 20 000 grains/m³ [127]. By using a constant level of allergen, differences in symptom severity during the study reflect differences in treatment efficacy and not differences in levels of allergen or exposure.

Studies in subjects with PAR require allergen challenge with, for example, HDM products. Usually, the allergen content of a room is defined by carpet and floor dust samples [128, 129]. However, HDM allergens can become airborne and the levels of Der p 1 (the major dust mite allergen) are important for sensitized individuals. Concentrations of 0.03–30 ng/m³ have been reported in different households, and the threshold value for sensitized subjects is around 10 ng/m³ [130]. Peak concentrations of 50–110 ng/m³ Der p 1 are used in ACCs [94, 110, 131], as an increased risk of bronchial attacks would be expected at levels above 130 ng/m³ [132]. However, owing to the instability of particles containing HDM allergens, simultaneous immunohistochemical analysis is necessary to confirm the allergenic load [132].

### Conducting clinical assessments

**Study design and target parameters.** ACC studies can be used for several evaluations, including:

- basic science, e.g. clinical relevance of mediator release in nasal secretions;
- priming effect, using repeated challenge sessions on consecutive days;
- exploring the time-course of symptom recovery following the end of allergen exposure;
- proof-of-concept for a compound in early Phase II;
- dose-finding for a new compound;
- onset of action of a single dose of medication;
- duration of action of a single dose of medication;
- efficacy during the late-phase allergic reaction;
- efficacy of a single dose of medication, compared with placebo and an active control;
- efficacy of a drug in steady state after 1 week of treatment, compared with placebo and an active control;
- comparison of efficacy among two or more rhinitis agents in the same class;
- safety and tolerability;
Table 9. Peer-reviewed publications on trials conducted in allergen challenge chambers in allergic rhinitis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Allergen</th>
<th>Trial design</th>
<th>N</th>
<th>Focus of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horak et al. [100]</td>
<td>Grass pollen</td>
<td>Three-way, double-blind, crossover</td>
<td>6</td>
<td>Onset and duration of the effects of astemizole, loratadine and terfenadine forte in rhinitis</td>
</tr>
<tr>
<td>Horak et al. [101]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>12</td>
<td>Efficacy, duration of action and dose-finding of a sustained-release dimethindene formulation in rhinitis</td>
</tr>
<tr>
<td>Horak et al. [102]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>7</td>
<td>Effects of astemizole on nasal obstruction in atopic patients</td>
</tr>
<tr>
<td>Horak et al. [103]</td>
<td>House dust mites</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>12</td>
<td>Controlled exposure to dust mite allergen in a dose-finding study of DMM in dust mite-allergic patients</td>
</tr>
<tr>
<td>Horak et al. [104]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>12</td>
<td>Dose-finding study of sustained-release DMM</td>
</tr>
<tr>
<td>Horak et al. [105]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, single-dummy, crossover</td>
<td>12</td>
<td>Efficacy (onset of action and duration of drug effect) and tolerability of astemizole-D and loratadine-D in rhinoconjunctivitis</td>
</tr>
<tr>
<td>Horak et al. [51]</td>
<td>Grass pollen</td>
<td>Randomized, placebo-controlled, double-blind, crossover</td>
<td>24</td>
<td>Quantification of conjunctival vascular reaction by digital imaging, using azelastine or placebo in grass pollen-allergic patients</td>
</tr>
<tr>
<td>Day et al. [106]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, placebo-controlled, parallel group</td>
<td>85</td>
<td>Onset of action of aerosolized triamcinolone acetonide nasal spray in seasonal allergic rhinitis</td>
</tr>
<tr>
<td>Donovan et al. [85]</td>
<td>Ragweed pollen</td>
<td>Controlled</td>
<td>43</td>
<td>Efficacy of &gt;2 years of ragweed immunotherapy for preventing rhinitis symptoms, compared with non-ragweed-allergic subjects or ragweed-allergic subjects who received no immunotherapy</td>
</tr>
<tr>
<td>Kyrein et al. [107]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>12</td>
<td>Efficacy of intranasally applied DMM solution as spray, compared with placebo and azelastine, in seasonal allergic rhinitis</td>
</tr>
<tr>
<td>Day et al. [108]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, placebo-controlled, parallel group</td>
<td>111</td>
<td>Onset of action and efficacy of terfenadine, astemizole, cetirizine and loratadine in seasonal allergic rhinitis</td>
</tr>
<tr>
<td>Day et al. [109]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, single-dose, placebo-controlled, parallel-group</td>
<td>99</td>
<td>Onset of action, efficacy and safety of a single dose of fexofenadine hydrochloride or placebo in seasonal allergic rhinitis</td>
</tr>
<tr>
<td>Day et al. [46]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, placebo-controlled, parallel-group</td>
<td>202</td>
<td>Efficacy and onset of action of cetirizine, loratadine or placebo in seasonal allergic rhinitis</td>
</tr>
<tr>
<td>Horak et al. [110]</td>
<td>House dust mites</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>24</td>
<td>Efficacy and safety of an oral formulation of cetirizine with sustained-release pseudoephedrine, relative to placebo, in perennial rhinitis</td>
</tr>
<tr>
<td>Horak et al. [111]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>24</td>
<td>Efficacy and tolerability of azelastine eye drops against pollen-induced allergic conjunctivitis</td>
</tr>
<tr>
<td>Horak et al. [112]</td>
<td>Birch pollen</td>
<td>Randomized, double-blind, placebo-controlled, parallel group</td>
<td>41</td>
<td>Efficacy and tolerability of short-term immunotherapy with sublingual birch pollen extract in rhinoconjunctivitis</td>
</tr>
<tr>
<td>Horak et al. [50]</td>
<td>House dust mites</td>
<td>Double-blind, placebo-controlled, crossover</td>
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<td>Randomized, double-blind, placebo-controlled, parallel-group</td>
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<td>Comparative onset of action and symptom relief with cetirizine, loratadine or placebo in seasonal allergic rhinitis; methodological validation study</td>
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<td>Investigation of whether quality of life indices are predictive of placebo and medication response to treatment for allergic rhinitis in a study of budesonide and placebo</td>
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<td>Randomized, double-blind, comparative, crossover</td>
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<td>Randomized, double-blind, two-treatment, two-period crossover</td>
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<td>Efficacy and safety of ketotifen eye drops as adjunctive therapy to mometasone nasal spray in seasonal allergic rhinoconjunctivitis</td>
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<td>Randomized, double-blind, crossover</td>
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<td>Efficacy and safety of exposure to four different concentrations of pollen in patients with seasonal allergic rhinitis</td>
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<td>Randomized, double-blind, parallel-group, placebo-controlled</td>
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<td>Ragweed pollen</td>
<td>Randomized, single-dose, double-blind, placebo-controlled, parallel group</td>
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<td>Onset of action, efficacy and safety of fexofenadine, 60 mg/pseudoephedrine, 120 mg, vs. placebo</td>
</tr>
<tr>
<td>Day et al. [120]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, placebo-controlled, parallel group</td>
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<td>Duration of effect of cetirizine and fexofenadine in seasonal allergic rhinitis</td>
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<td>Day et al. [124]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, parallel-group, placebo-controlled</td>
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<td>Efficacy and onset and duration of action of levocetirizine and desloratadine in seasonal allergic rhinitis</td>
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<td>Stübner et al. [121]</td>
<td>Grass pollen, house dust mites</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>112</td>
<td>Efficacy and onset of action of levocetirizine and loratadine in patients with seasonal or perennial allergic rhinitis</td>
</tr>
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<td>Horak et al. [122]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>94</td>
<td>Onset and duration of action of levocetirizine and fexofenadine in patients with seasonal allergic rhinoconjunctivitis</td>
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<td>Zieglmayer et al. [97]</td>
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<td>Efficacy and safety of cetirizine plus pseudoephedrine and budesonide nasal spray in patients with seasonal allergic rhinitis</td>
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<td>Krug et al. [123]</td>
<td>Grass pollen</td>
<td>Randomized, double-blind, parallel-group, placebo-controlled</td>
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<td>Efficacy and safety of loteprednol etabonate nasal spray in patients with seasonal allergic rhinitis</td>
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<td>Day et al. [125]</td>
<td>Ragweed pollen</td>
<td>Randomized, double-blind, placebo-controlled</td>
<td>599</td>
<td>Efficacy 5–12 h postdose of cetirizine and fexofenadine in seasonal allergic rhinitis</td>
</tr>
</tbody>
</table>

DMM, dimethindene maleate; o.d., once daily.
short-term change in QoL parameters; (The impact of symptoms on QoL parameters, as well as the ability of subjects to concentrate and perform activities, will be influenced both by the allergic reaction and by the effect of the trial drug, which can be measured with appropriately designed questionnaires [90].)

The number and duration of challenge sessions carried out for a trial will vary. As many of the studies to date have focused on determining onset of action, the duration of challenge sessions has generally been restricted to one to two sessions conducted over 1–2 days – this is sufficient for determining the onset and duration of action of a single dose of medication. However, it is feasible for protocols to involve a greater number of sessions separated in time: for example, in a multiple-arm crossover study [87]. A balanced crossover study can provide precise results with few subjects. In the VCC, 28 subjects have been found to give sufficient power in a two-arm crossover trial, but for a comparison of two or more active arms and placebo, the number of subjects must be increased accordingly.

Subjects are typically exposed to allergens for between 4 and 10 h in an ACC study, which is consistent with daily exposure to pollen in a ‘real life’ setting. Data from trials investigating allergen exposures of up to 8 h in the VCC have shown that subjects’ symptoms remain stable throughout this time-period, allowing extended evaluation of medication efficacy [50].

Medication may be administered before or after the allergen challenge, depending on whether a treatment is intended to be taken prophylactically or as needed. However, in most cases, subjects ingest medication approximately 2 h following the beginning of antigenic challenge, if they achieve a predetermined qualifying symptom level. This baseline level of symptoms varies between studies, depending on the selection criteria specified by the protocol. The range of symptoms (mild, moderate or severe) selected for in the study population is important, so that if the treatment taken is effective, a decrease in symptoms can be observed.

Figure 6 shows an example of a study session in an ACC [46, 47]. Subjects remain in the EEU, in a pollen-containing environment, for 2 h to establish a baseline symptomatic state. The first dose of the trial medication(s) or placebo is administered and subjects are monitored regularly for the next 5 h. Subjects return the following day so that an end-of-dose (24 h) assessment of symptoms can be carried out before a second dose of medication or placebo is given.

The frequent, strictly timed evaluation of symptoms means that from the initiation of treatment until the 24 h period of expected efficacy, ACCs are one of the more clinically relevant methods to determine when the drug takes effect, as well as the duration of action and efficacy at other time-points over that period. ACCs are also well suited to the conduct of dose-ranging studies and proof-of-concept evaluations of medications in early clinical...
development, as well as for direct comparisons of two or more medications.

Data collection and analysis. ACCs enable subjects' symptoms to be assessed separately and monitored from their inception until resolution. Missing values occur only where subjects discontinue a crossover trial or withdraw early following randomization.

On entering the chamber, subjects complete baseline symptom assessments. Depending on the chamber, subjects may record information directly into a computer (as in the VCC), or onto diary cards that can be read by an optical card reader (as in the Kingston EEU). Under the supervision of medical personnel, subjects then complete further self-assessments at regular intervals, depending on protocol requirements (e.g. every 15–30 min), rating the nature and severity of their symptoms. Subjects record the symptoms experienced at each time-point, with no recall or retrospective assessment required.

Subject-rated scores are still preferred as the primary measure of efficacy in AR trials [3]. However, in order to gather additional efficacy data from each session, objective measurements can be collected. It is possible to test skin response, lung function, nasal airflow, cellular mediators, blood levels of immune cells, nasal cytology or nasal secretion depending on the desired efficacy endpoints. An advantage of the single-centre ACC setting is that the same apparatus and technique can be used to conduct these tests in all subjects; rhinomanometry, for example, is hampered in traditional trials by a diversity of instruments and procedures in multicentre settings. In an ACC, these examinations can be completed at a predetermined time, thereby allowing more accurate comparison between study groups.

Outcome measures are defined precisely in the protocol for each study conducted. Examples of outcome measures that can be achieved from ACC trials include the following:

- change in total symptom complex or individual symptom scores at a certain time-point, or over a period of several hours;
- time to onset of action;
- time to onset of ‘clinically important’ symptom relief;
- time to maximal effect;
- degree of symptom relief at 24 h/end of dosing interval;
- changes in respiratory parameters at 24 h (objective measures);
- changes in mediator release in nasal secretion (objective measures);
- the number needed to treat [133] for one subject to benefit;
- global evaluation of willingness to take medication again;
- global evaluation of satisfaction with treatment;
- change in QoL parameters;
- incidence of adverse events during entire treatment period.

The need to validate new methodology

For a given clinical trial, assumptions are made and limitations imposed, in terms of the inclusion/exclusion criteria used to enrol subjects, the conditions of medication administration and the methods of assessing response. In order to make clinical interpretations, it is necessary to assess the accuracy of results obtained in an ACC and to compare these with findings from other trial settings. There is a need to ensure that processes are consistent, results can be reproduced, comparisons can be made across trials and that increasing system complexity does not also increase the error rate. Additionally, validation of clinical findings compared with other standard methodologies is required to ensure scientific integrity of clinical data and to meet regulatory guidelines. Individual chambers should have their own supporting validation documentation. The VCC and the EEU in Kingston have been found to provide accurate and reproducible results, and to be valid instruments to assess the efficacy of anti-allergic medications [46, 47, 86, 87, 121, 134].

There are three main areas that require validation:

- the ACC itself;
- the computer systems used for capture and analysis of the data;
- the clinical trial processes used to ensure accuracy and reproducibility of results.

Fundamental to the ACC methodology are the following points, which will be described in more detail in the following sections:

- the intra- and inter-study allergen concentration should be constant;
- clinical findings in an ACC should be consistent when ACC protocols are repeated.

Constancy and reproducibility of allergen concentration. Allergen concentration is the most important parameter in the validation of a chamber because the principle that subjects are exposed to a well-defined stimulus is fundamental to the methodology. The spatial and temporal distribution of the allergen must be constant, giving equal exposure to all subjects for the duration of the challenge session. The allergen concentration varies widely in studies conducted in the natural environment, and reproducibility of conditions is impossible. The EEU and VCC have repeatedly demonstrated that a highly reproducible allergen level is attained not only between participating subjects within a cohort, but also for each cohort over the course of each study.
The following figures and tables provide examples to demonstrate that the allergen concentration:
- remains constant throughout the course of a single challenge session (Figs 7 and 8);
- can be reproduced within prespecified tolerance limits in subsequent sessions (Fig. 8 and Table 10);
- is maintained at a constant level, in contrast to variable allergen concentrations observed in other study settings (Fig. 9).

**Reproducibility of clinical findings between studies.** If conditions are controlled tightly within an ACC, repetition of the same protocol on separate occasions would be expected to yield the same results. For active treatment arms, although there is some variability in the magnitude of responses in different studies, the comparative efficacy of each agent is highly consistent. Variability may be seen between placebo arms in repeated trials, but this is probably due to differences in study populations.

**Desloratadine versus placebo.** Two studies in the VCC investigated the effect of desloratadine on nasal congestion [86, 87]. In both studies, subjects received either desloratadine, 5 mg, or placebo for 7 days, followed by a 10-day washout period, before being crossed over to the other treatment for 7 days. Despite very small variations in the placebo response, the response to treatments is similar [86, 87]. In these studies, both the placebo and the medication data are highly reproducible across the studies (Table 11).

**Cetirizine versus loratadine versus placebo.** In the Kingston EEU, a study investigating relief of ragweed-induced

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
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<td>98</td>
<td>1450</td>
<td>1330</td>
<td>1670</td>
</tr>
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</table>

**Fig. 7.** Fluctuation of grass pollen load (approximately 5%) during a 6 h allergen challenge session in the Vienna Challenge Chamber.

**Fig. 8.** Average 30 min ragweed pollen concentration in the Environmental Exposure Unit during separate study periods undertaken during the summer of 1998. Reproduced from Day and Briscoe [92], with permission.
AR symptoms with cetirizine, loratadine or placebo was conducted in August 1995 [46] and repeated in April 1999 [47]. The treatment schedule is shown in Fig. 6. Despite being conducted almost 4 years apart, the results were virtually identical in both studies. Although the treatment effect was slightly greater for all agents (including placebo) in the first study, the comparative efficacy was the same and the onset of action for cetirizine was 1 h compared with 3 h for loratadine in both studies (Table 12) [46, 47]. The only apparent difference between the two studies was the greater placebo response on day 2.

Symptoms in an allergen challenge chamber are similar to those obtained by other methods

Allergen challenge in an allergen challenge chamber results in a similar symptom score to that obtained in a park setting, but with less fluctuation. In a study in the VCC (F. Horak, personal communication), subjects were challenged for 4 h with a grass-pollen load of 2000 grains/m³, then given an ocular challenge test (OCT) with a grass-pollen allergen solution. After 2 months, the same subjects repeated the study, except that they were challenged in a park setting for 4 h instead of in the VCC, and then given an OCT. Pollen levels in each setting are shown in Fig. 9. The VCC and park settings yielded closely matched results with respect to ocular itching for the 4-h study period, with less fluctuation in symptom score in the ACC setting (Fig. 10).

In the same study, conjunctival digital imaging was used to assess the impact of allergen challenge in the VCC and the park setting (Fig. 11), and the results were consistent.

Symptom scores with placebo or treatment in an allergen challenge chamber are of similar relative magnitude to those obtained by an ocular challenge test. In a dose–finding study using azelastine eye drops, the OCT was confirmed as giving similar results to the VCC [111]. In this double-blind, randomized, placebo-controlled, crossover trial, 24 subjects with a history of AR were challenged out of season with a single dose of azelastine 0.025%, 0.05% or 0.1% eye drops. The drops were administered 60 min before a 4 h challenge session, and an additional OCT was

<table>
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<tr>
<th>Cetirizine, 10 mg</th>
<th>Loratadine, 10 mg</th>
<th>Placebo</th>
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<tr>
<td>Mean change in TSC from baseline (%)</td>
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<td>Study 2</td>
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<td>25.4</td>
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<tr>
<td>Loratadine</td>
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<td>-</td>
</tr>
<tr>
<td>Placebo</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Onset of action (h)</td>
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<td>Study 2</td>
</tr>
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<td>1</td>
</tr>
<tr>
<td>Loratadine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Placebo</td>
<td>-</td>
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</tr>
</tbody>
</table>

Table 12. Comparison of key findings in a protocol repeated within the Kingston Environmental Exposure Unit, in two studies conducted 4 years apart [46, 47]

TSC, total symptom complex.

In a dose–finding study using azelastine eye drops, the OCT was confirmed as giving similar results to the VCC [111]. In this double-blind, randomized, placebo-controlled, crossover trial, 24 subjects with a history of AR were challenged out of season with a single dose of azelastine 0.025%, 0.05% or 0.1% eye drops. The drops were administered 60 min before a 4 h challenge session, and an additional OCT was

Table 11. Reproducibility of symptom scores across two consecutive studies in the Vienna Challenge Chamber using identical protocols but different subjects [86, 87]

<table>
<thead>
<tr>
<th></th>
<th>0–2 h</th>
<th>0–6 h</th>
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<td>Active</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>Study I</td>
<td>Study II</td>
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<td>Nasal secretion weight (g)</td>
<td>0.53</td>
<td>0.5</td>
</tr>
<tr>
<td>Rhinorrhea score</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Total nasal score</td>
<td>6.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Total non-nasal score</td>
<td>2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

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administered at the end of the session. There were 4 study days, each separated by a 2-week washout period. The use of 0.05% azelastine for the treatment of allergic conjunctivitis was supported by the sum of the VAS scores for itching of the eyes [Fig. 12] and lacrimation [Fig. 13], and the degree of vascularization (data not shown) obtained from VCC and OCT methodology [111].

**Criticisms of allergen challenge chambers**

As with other experimental models used to study AR and its treatment, the ACC model has been subject to critical analysis from the scientific community. Actual and hypothetical criticisms are indicated and addressed in the following section.

**Allergen challenge chambers do not represent ‘real life’ conditions.** Despite their ability to provide highly controlled efficacy, safety and pharmacological data, studies conducted in ACCs have been criticized as not representing ‘real life’ conditions, leading to debate about whether conclusions drawn from these trials are clinically relevant. While allergic subjects participating in ACC studies are not exposed to the sensitizing allergen as in their natural, ‘real life’ environment, they regularly report that symptoms elicited in the EEU, an ACC setting, are similar to those experienced during the pollen season. This observation was confirmed recently in a survey in which subjects completed an assessment of their allergic symptoms in ragweed season, and again while participating in a trial conducted in the EEU [135]. Additionally, the activities that subjects engage in when in the EEU are similar to those encountered in a ‘real life’, ‘at home’ setting: subjects are free to watch movies, read or perform other activities while seated. Subjects may also briefly leave the seating area to stretch, obtain food and refreshments, and go for toilet breaks [44].

A single allergen source is used in an allergen challenge chamber. As a number of allergens and environmental factors may contribute to an individual’s development of rhinitis, it has been argued that the single allergen exposure that is typically used in an ACC setting may not reflect the natural pathological process. At the molecular level, however, AR is initiated by the interaction of allergen with specific IgE. This is followed by immediate histamine release and then by other cellular events, which result in the clinical manifestation of AR regardless of the type or number of allergenic triggers. An atopic individual may develop AR as a result of single or
multiple allergen sensitivity. Therefore, it follows that the results obtained on the efficacy of anti-allergic medications derived from ACC studies using one allergen are transferable for the treatment of AR caused by sensitivity to different allergens. Additionally, as a single allergen can produce the full spectrum of symptoms required to evaluate an anti-allergic medication, the logistics of presenting multiple allergens would complicate a study unnecessarily.

Seasonal priming does not occur in an allergen challenge chamber. An allergic individual’s reactivity to a seasonal allergen should increase within a season because of the priming effect [21]. Additionally, priming by one allergen appearing early in the season may induce sensitivity to another allergen appearing later in the season. It is argued that the priming effect is overlooked in ACC studies when they are conducted out of season. This leads to the assumption that the reactivity of participants to allergens in an ACC setting may differ from that observed in a natural environment.

However, ACC studies are not limited by season and may be conducted at any time of the year, for both non-seasonal and seasonal allergens. Subjects enrolled in ACC studies, like traditional studies, have a well-documented history of SAR and/or PAR (where applicable), are screened for sensitivity to the test allergen by skin testing and are then exposed to the allergen at a predetermined level in the priming phase in order to activate allergic reactivity. Only those subjects who demonstrate adequate symptomatology required by a study protocol are later challenged and randomized to receive double-blind medication. As long as the allergen is being delivered, the priming effect persists throughout a study [99].

During crossover trials at the University Clinic VCC, a washout period of 8–10 days between individual challenge sessions is often included to avoid the priming effect within a trial. However, the priming phase is an important element in the study design of all EEU (Kingston) studies and is included whether studies are undertaken in or out of season. Reactivity to the challenge allergen awakens dormant responsiveness in the priming phase, leading to adequate symptoms on the study date.

As in traditional or park studies, ACC studies may be influenced by both seasonal and non-seasonal priming. With all three types of study, the degree of a subject’s exposure to other sensitizing allergens is variable, as the level of previous environmental exposure cannot be controlled. Priming is a complex process that occurs in nature and in the ACC setting, and manifests as typical allergy symptoms. The rate and severity of symptom development during priming is a phenomenon that is currently being studied in the ACC setting [24].

The allergen concentration in an allergen challenge chamber is not ‘natural’. There is concern that the levels of allergen maintained in an ACC setting are higher than those experienced by allergic individuals in their ‘natural’ environment. Daily environmental pollen levels that are reported as a 24 h average are obviously downwardly influenced by the pollen-low hours of the night. Furthermore, pollen service lines measure pollen from a height of 15 m. However, pollen concentrations at 1.5 m above the ground have been found to be much higher, and, therefore, the daily average count from a service line must be multiplied by 11–26 to compensate for these differences [136], which are compounded by the reality that service line readings are usually taken far from trees and other local pollen sources [137].

A concentration of 2000 grass pollen grains/m^3 air used in the VCC corresponds with 75–180 grass pollen grains/m^3 air measured as a daily mean concentration at a height of 15 m. Concentrations in excess of 1000 grass pollen grains/m^3 have been reported at 20 m, which

Fig. 13. Sums of visual analogue scale (VAS) scores for lacrimation at each time-point per treatment during the Vienna Challenge Chamber (VCC) sessions and 15 min after ocular challenge test (OCT). Reproduced with permission [111].
indicate higher counts at ground level [65]. In the EEU setting, a target ragweed pollen concentration of 3500 ± 500 grains/m³ is consistent with peak outdoor levels reported by others [71, 138]. Thus, allergen concentrations in an ACC are comparable with peak environmental levels found during the pollen season.

Dose-dependent relationships between the total nasal symptom score, nasal flow rate and nasal secretions with pollen levels ranging from 1000 to 8000 grains/m³ have been observed by Krug et al. [88] in their ACC. These are at levels in or above the upper range of naturally occurring grass pollen. As symptom levels are known to fluctuate with changes in pollen levels, traditional trials are scheduled during the peak of the pollen season when symptoms should be present over the duration of the study [81]. The relationship between allergen concentration and symptom induction highlights the importance of using pollen levels within upper environmental ranges to facilitate sufficient symptom induction. ACC studies can emulate the peak of the allergy season, which is only encountered irregularly in traditional and park studies and, in this context, the US FDA has indicated that ACC studies may be used to evaluate anti-allergic medications for the prophylactic treatment of SAR [3].

The diurnal variation in pollen levels existing in nature is not seen in a controlled setting, and this has been challenged as a potential limitation of ACC studies. However, diurnal variation in pollen levels inherent in park studies has led to concerns about irregularity in symptom intensity [71]. In this respect, different times of onset of action were reported for the same treatment, at sites that had varying levels of pollen in a multisite park study [75], leading to the speculation that differing levels of pollen were responsible. The EMEA requires the degree of exposure of subjects to allergens during the study to be documented, to address the possibility that an improved symptom score may be due to spontaneous improvement, or declining or absent allergen exposure rather than treatment effect [4]. Both the EMEA and the FDA indicate that for multicentre SAR trials, pollen levels should be measured at each study centre [3, 4]. The significant shortcoming of variable pollen exposure in traditional trials and park studies is eliminated in ACC studies: for a given study population, which may be comprised of multiple cohorts, each cohort and each subject within a cohort is predictably exposed to the same levels of pollen, providing the desired consistency of pollen exposure for studies involving both small and large numbers of subjects.

**Subjects in an allergen challenge chamber are affected by the trial context.** It has been suggested that subjects participating in ACC studies, like those in park studies, interact with other trial subjects, which may influence their experience or reporting of symptoms. Results obtained in ACC studies have been shown to be reproducible and to discriminate between treatment and placebo, indicating that subjects record their symptoms accurately. This is exemplified by two EEU trials, identical in design but conducted years apart, which evaluated the comparative onset of action of cetirizine and loratadine. In the first trial, undertaken in 1995, 202 allergic subjects were exposed to ragweed pollen for 7 and 6 h, respectively, on 2 consecutive days, where they were randomized to receive daily doses of cetirizine, 10 mg, loratadine, 10 mg or placebo [46]. Cetirizine produced a 37.4% mean reduction in MSC scores vs. 14.7% with loratadine, and 6.7% with placebo [46]. Onset of action, as assessed by a reduction in MSC and TSC scores vs. placebo, was evident within 1 h for cetirizine and 3 h for loratadine. The second study, which included 360 subjects, was completed in 1999, and showed comparable efficacy and identical onset of action [47].

Another concern raised against the trial context in which subjects are gathered together in a similar environment is the likelihood of greater placebo response because of the expectation of a beneficial effect of therapy [81]. Subjects are made aware during the consent process, as well as at other times over the duration of the study, of the random allocation of placebo or treatment to each and every participant. Placebo response rates in ACC trials have been found to be comparable with those observed in traditional trials [139, 140], but have also been found to differ depending on whether treatment is applied orally, topically or by injection [113, 122], which should also be considered in the trial design.

**Subject demographics are limited in allergen challenge chamber studies.** A limitation of ACC studies is that the pool of subjects available to participate is restricted to the catchment area of existing ACCs. This could be a problem in geographic regions with a limited ethnic population and, where it is, this could be addressed by including university students or deriving study participants from a nearby major city.

**Allergen challenge chamber studies are of short duration.** ACC studies have typically been designed to evaluate the onset and duration of action of anti-allergic treatments. This information is essential to the efficacy of anti-allergic medications as determined by the ARIA/EAACI workshop group [141]. Based on the protocols, the duration of these studies has generally been relatively short, usually 1–2 days, and therefore these studies are not readily suited nor intended to explore extended long-term efficacy or safety of anti-allergic medications. However, ACC studies have also been designed to evaluate medications for protracted periods of time (i.e. 1–2 weeks), with subjects taking medication on a daily basis [86, 106, 123]. The duration of ACC studies can be readily extended to provide medication efficacy and safety data at a steady...
state, whereby subjects are exposed to consistent levels of allergen on designated dates over the treatment period, without the variable allergen loads associated with park studies or traditional studies. In this type of study, subjects take the trial treatment at home and return to the ACC setting for subsequent allergen challenge in the coming days or weeks. An example of this kind of study has been published recently by Krug et al. [123] (Fig. 14).

The inflammatory stage of the allergic reaction can be readily studied in an ACC, in which long-term studies may be designed to evaluate pathophysiological events in chronic AR. The effect of medications on chronic symptoms can be explored in subjects sensitive, for example, to HDMs, as this perennial allergen occurs on a daily basis, year round, producing symptoms consistent with chronic exposure.

**Summary and discussion**

ACCs provide a valuable method for assessing anti-allergic treatments in a controlled and reproducible environment. The role of ACCs in the evaluation of new drugs being considered for development, as well as in comparative efficacy of approved anti-allergic medications is expanding. The increase in peer-reviewed publications reporting results of well-controlled ACC trials has led to discussion about the use of ACCs in a wider range of trial designs. At present, there are at least six established ACCs worldwide, three in North America and three in Europe; two ACCs are under development in Japan.

A better understanding of the capabilities of the ACC and its performance relative to traditional studies and other experimental models (e.g. NPTs and park studies) is needed for greater recognition by regulatory and scientific communities. Even with the large body of studies available in the current literature, a direct comparison of the various models is difficult because of the differing study designs used, including varied symptom scoring scales, timing of assessments, efficacy parameters and statistical plans. Nevertheless, a prospective comparison using each model in a similar study design would facilitate a rigorous analysis of their strengths and weaknesses and contribute to a better understanding of their capabilities [44]. The next step is the expanded use of the ACC in the evaluation of anti-allergic medications at steady state, in longer-term studies.

As indicated, the ACC offers many advantages and is especially suited to the utilization of the full spectrum of available methodologies for the assessment of treatment efficacy. This versatility is particularly advantageous for the exploration of new endpoints and innovative techniques, such as facial thermography [142, 143], which cannot be readily carried out in park or traditional studies. The combination of the controlled and reproducible environment of an ACC, together with its clinical relevance, will continue to contribute to a greater understanding of AR and its treatment.

**Conclusions**

Traditional outpatient studies are the accepted method of evaluating the efficacy of anti-allergic treatments for AR, as they represent the normal environment in which allergic subjects are exposed to allergen and treated for symptoms. These studies also address safety and measures of efficacy because of their long duration.

The variability of pollen counts in traditional outpatient SAR studies is given extensive consideration in the EMEA guidelines [4]. These guidelines acknowledge that the degree of allergen exposure may impact on efficacy and...
that documentation of subjects’ exposure to the relevant allergens is required to help determine whether an improved symptom score is a result of treatment effect, spontaneous improvement or insufficient allergen exposure. The rationale and requirement for a controlled environment is, therefore, substantiated. The clinically relevant ACCs have been established as a means to assess anti-allergic medications, based on their capacity to control a number of variables associated with traditional studies. Important parameters, such as onset and duration of action, as well as other precise measures of efficacy, are effectively determined with this method. This role in defining the onset of action of anti-allergic medications is recognized by the FDA [3], who also acknowledge the use of ACC studies in the evaluation of anti-allergic medications for the prophylactic treatment of SAR. Nonetheless, the role of ACCs, as acknowledged by regulatory bodies, remains secondary compared with traditional trials. The pharmaceutical industry also recognizes the value of ACC studies beyond their ability to evaluate precise measures of efficacy, including the requirement for fewer subjects over a shorter period of time because of the single-centre design of ACCs compared with traditional studies, with evident ethical advantages and financial savings.

This paper has reviewed the studies and physical features of ACCs and other methods to enable informed assessment of these models for the evaluation of treatments for AR. Highlights include the unique capability of ACC studies to produce and closely monitor symptoms from onset to resolution, and to implement methodologies for the objective measurement of efficacy when desired. The variability in inter-individual allergen exposure is eliminated, compliance with study drug is improved, and there is reliable and timely completion of symptom assessments.

When ACC studies of similar design are repeated, both in and out of season, and years apart, consistent outcomes are observed. Even though challenges are undertaken in an enclosed space because of the need to control allergen levels, the setting is conducive to subjects and researchers alike, and subjects readily equate symptoms to those experienced in the outdoors. In the case of the ACCs using pollen as the sensitizing allergen, the levels selected are intended to represent peak levels that are ordinarily observed outside during the pollen season. For example, the levels of ragweed pollen chosen for EEU studies are well within the outdoor range of peak levels and have the effect of producing the full spectrum of symptoms, from mild to severe, required to test anti-allergic treatments adequately. The ACC can also be adapted readily to long-term studies, in which measures of QoL and safety can be determined and important pharmacodynamic information obtained. There is much opportunity for further application of ACCs in the study of AR and its treatment, and this can be realized by innovative approaches that recognize the potential of these systems.

Acknowledgements

This supplement was sponsored by UCB Farchim SA, Switzerland. Editorial assistance was provided by Emma James and Jenni Macdougall, Caudex Medical Ltd. The assistance of Jodan Ratz and Elizabeth Rafeiro is gratefully acknowledged.

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