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Controlled Allergen Challenge Facilities and Their Unique Contributions to Allergic Rhinitis Research

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Abstract The aim of this study is to review advances in basic and clinical allergic rhinitis (AR) research over the past decade that have been conducted using controlled allergen challenge facility (CACF) models of allergen challenge. Databases, including PubMed, Medline, and Web of Science were searched for articles employing an ambient pollen exposure in a controlled facility to study AR, published between 2004 and the present date, using the terms as follows: CACF, Environmental Exposure Unit (EEU), Vienna Challenge Chamber (VCC), Fraunhofer Institute Environmental Challenge Chamber, Atlanta Allergen Exposure Unit, Biogenics Research Chamber, Allergen BioCube, Chiba and Osaka Environmental Challenge Chamber, exposure unit, challenge chamber, or environmental exposure chamber. Articles were then selected for relevance to the goals of the present review, including important contributions toward clinical and/or basic science allergy research. CACFs offer sensitive, specific, and reproducible methodology for allergen challenge. They have been employed since the 1980s and offer distinct advantages over traditional in-season multicentre trials when evaluating new treatments for AR. They have provided clinically applicable efficacy and pharmacologic information about important allergy medications, including antihistamines, decongestants, antileukotrienes, immunotherapies, and nasal steroids. CACF models have also contributed to basic science and novel/experimental therapy research. To date, no direct studies have been conducted comparing outcomes from one CACF to another. Over the past decade, CACF models have played an essential role in investigating the pathophysiology of AR and evaluating new therapies. The future opportunities for this model continue to expand.

Keywords Controlled allergen challenge facilities · Allergic rhinitis · Immunotherapy · Nasal steroid · Antihistamine · Pollen

Abbreviations

AR	Allergic rhinitis
CACF	Controlled allergen challenge facility
CRTH2	Chemoattractant receptor homologous molecule
EEU	Environmental exposure unit
EPR	Early phase responders
SLIT	Sub-lingual immunotherapies
TNSS	Total nasal symptom score
TRPV1	Transient receptor potential vanilloid 1
VCC	Vienna challenge chamber

Introduction

Allergic rhinitis (AR) is an IgE-mediated nasal disorder involving inflammation and hyperactive nasal mucosa, resulting in symptoms of rhinorrhea, sneezing, nasal pruritus, congestion, and aggravation of comorbid asthma [1•]. The prevalence of AR is increasing, currently affecting approximately 10–25 % of the population worldwide [1•]. In recent years, the

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use of controlled allergen challenge facilities (CACFs) (also referred to as "exposure units" or "challenge chambers") has contributed to our understanding of the pathophysiology of AR and pharmacological properties, efficacy, and onset of action of new therapies. Treatments investigated using CACF models include antihistamines, leukotriene modifiers, corticosteroids, and immunotherapy [2••]. Herein, we will review advances made in basic and clinical allergy research through the use of CACF models. Studies published in abstract form only are not discussed, due to space constraints.

Brief Historical Context

The first multi-participant CACF was the Vienna Challenge Chamber (VCC; Vienna, Austria), described by Horak and Jäger in 1987 [2••, 3••]. Shortly thereafter, Day and Clark adapted their specially engineered particulate distribution technology (originally used to study urea formaldehyde in 1981), for pollen distribution, creating the Environmental Exposure Unit (EEU) at Kingston General Hospital, Kingston, Ontario, Canada [4–6]. In Europe, Rønborg et al. constructed an allergen exposure facility in Copenhagen, Denmark, and validated it for use with house-dust mite allergen [7•]. The Fraunhofer Institute Environmental Challenge Chamber (ECC) was established in Hannover, Germany in 2003 [8••]. Across North America, facilities have emerged, including the Atlanta Exposure Unit [9–11], the Environmental Exposure Chamber (EEC) [12, 13], the Biogenics Research Chamber [14, 15], and the Allergen BioCube [16]. In Japan, the Environmental Challenge Chamber at Chiba University was built in 2008 [17•], followed by another allergen challenge chamber in Osaka [18]. An Allergen Challenge Theatre has been described in Ottawa, Ontario, but only in abstract form [19]. These facilities were uniquely and independently developed, and employ various technologies to achieve allergen distribution, monitoring, and air quality control/assessment.

Brief Technical Overview and Considerations

CACFs are custom-designed rooms that house study participants in a controlled environment, in which exposure to consistent allergen levels can take place and symptoms can be monitored [2••]. Studies conducted to date have been monocentric, and there are technical differences between units, the most palpable being the method of allergen dispersion and allergen concentration monitoring [2••, 3••].

In the VCC, clean air and a second air circuit carrying allergen-loaded air, enter through the ceiling and are exhausted at floor level [2••, 3••]. Modified Burkard pollen traps are employed to monitor allergen levels [2••, 3••]. The EEU employs a laser-aided pollen dispersion system and directional fans to propel the pollen over the participant seating area [6]. Rotorod samplers, distributed at seven locations, collect pollen at 30-min intervals, which correspond to the participant symptom scoring times [6]. The EEU employs slightly negative pressure to minimize pollen loss to the rest of the hospital, and the ventilation system uses high efficiency filtered outdoor air, with temperature and humidity control, exhausted directly back into the outdoors [6]. The Atlanta Allergen Exposure Unit similarly employs filtered outdoor air and Rotorod samplers [2••, 9].

At the Fraunhofer Institute, HEPA-filtered air enters via swirl inlets and thermal convection from floor heating aids in mixing [8••]. Pollen is introduced via a feeding system and a pneumatic dispersion nozzle located on the floor above the unit [8••]. Pollen concentrations are monitored by a laser particle counter, and every 30 min by two Rotorod samplers [8••]. Pollen in the Chiba environmental challenge chamber is also introduced from reservoirs above the ceiling, where the particles are agitated by fans to fall and drop down through holes into the participant seating area [17•]. Levels are monitored at 56 points using automatic pollen counters, including one on the back of each participants' chair [17•]. Although the automated counters are not pollen-specific, they have been validated against Durham samplers [20]. Other designs exist; however, the facilities above have published the largest number of manuscripts detailing technical operations. While individual CACFs exhibit differences, it is generally agreed that well-documented, uniform concentration of allergen, selected to be relevant to levels found outdoors, is the most important constant in CACF systems [2••], and a lack of such control would generate concerns when interpreting the study outcomes.

Advantages of the CACF Model

CACFs offer advantages over traditional in-season multicentre trials when evaluating new AR treatments. Logistical problems are presented by traditional studies that are scheduled to coincide with peak pollen season, as levels can be influenced by weather, highly variable between sites and consecutive seasons, significantly impacting the apparent efficacy of treatment [21]. Furthermore, individual participants' exposures are affected by lifestyle [22], adherence to scheduled administration of study medication, and symptom recording cannot always be ensured [6, 23]. CACFs were developed to address these variables, while recapitulating many characteristics of an outdoor allergy study [2••, 6, 23]. Although to date, the burden of proof for determining the efficacy of new treatments remains with in-season outpatient trials, expert reports, the European Medicines Agency and the proceedings of an Advisory Committee of the US Food and Drug Administration (FDA) Meeting underscore the utility of

CACFs, and perhaps they will contribute to the regulatory determination of efficacy in the future [24–27].

Reproducibility of CACF Findings

Reproducibility of findings between studies is an important consideration in clinical research. If conditions are tightly controlled, repetition of the same protocol on separate occasions should yield the same results. Indeed, both the VCC and the EEU have demonstrated this capability. Consecutive studies in the VCC determined the efficacy of desloratadine on nasal congestion and demonstrated similar results [28, 29]. In the EEU, two studies, separated by approximately 4 years, investigated the effects of cetirizine or loratadine vs. placebo in the treatment of seasonal AR, and yielded almost identical comparative efficacy results [30, 31]. Thus, placebo and medication responses are highly reproducible in CACF studies.

Priming and Other Factors That Affect the AR Symptom Response

Despite the reproducibility of CACF clinical findings, there are known factors that can affect an individual's symptoms upon exposure. The priming effect is defined as an increase in reactivity of the nasal membrane following repeated exposure to allergen [32••]. This well-recognized effect of repeated allergen exposure in CACFs has become somewhat of an advantage of the model. In CACF clinical trials, priming sessions are often used to increase sensitivity to a specific allergen by inducing mucosal inflammation, facilitating the development of robust symptoms and boosting participant inclusion [6]. In the EEU, priming has been shown to standardize pre-dose symptoms and greatly reduce symptom variability [6, 23]. In the Osaka allergen challenge chamber, a single challenge to Japanese cedar pollen has been proven to induce robust symptoms at the end of pollen season, while three consecutive challenges are required out of season [33].

Important individual factors that affect EEU studies include sensitization to dust mite, dog, or grass, current exposure to dog or cat, and rhinitis-related quality of life [34•]. Thus, in the EEU, perennial allergens have been demonstrated to be confounders under certain conditions. Recently, Jacobs et al. showed that pollen sensitization can conversely become a confounder when perennial allergens are being studied, demonstrating that pollen allergy affects symptom development to house dust mite [35]. Quality of life at study entry also represents an important consideration, affecting response to placebo [36]. As CACF studies, by their nature, induce disease symptoms, participation in CACF trials can, in turn, have effects on an AR participants' quality of life [37•]. While the above factors, related to the individual research participant, appear to be separate from induced priming, they may collectively reflect the importance of endogenous priming in the participant's day-to-day life.

Effects of, and Comparison to, the Natural Pollen Season

Due to the effects of natural exposure on participants' symptom responses, traditionally, CACF studies have been conducted outside of the relevant pollen season. However, scientists from the Fraunhofer Institute demonstrated an identical treatment effect of cetirizine/pseudoephedrine, compared with placebo, both within and outside of the pollen season [38••]. The CACF model also offers better reproducibility and sensitivity/specificity of total nasal symptom score (TNSS), compared to two measures during one natural pollen season [39]. In the Biogenics Research Chamber, concordance between induced allergic symptoms and those experienced during the natural season has been shown [40•]. Multiple allergic sensitizations have also been shown to result in "pre-priming" during the natural season [41]. Therefore, in general, CACF models achieve similar symptoms to those experienced by AR sufferers during the natural pollen season, but pre-priming must be taken into consideration, as they can occasionally affect results.

CACF models are also capable of recapitulating different phenotypic responses to allergen exposure observed in the "real world". Recently, it has been shown that participants' AR responses can be clearly phenotyped into isolated early phase responders (EPR), dual responders (early and late phase), as well as an intermediate protracted EPR phenotype, using the EEU [42]. Significant differences were evident between EPR, protracted EPR, and dual responders beginning 3 and 8 h after a 3 h ragweed pollen exposure, respectively [42]. This study demonstrated that the protocols and technology employed in CACF models allow for the phenotyping of participants based on AR symptoms during and after pollen exposure [42].

Oral Antihistamines

Over the past decade, CACF models have contributed extensively to our understanding of the efficacy, onset of action, and duration of action of various antihistamines. Traditionally, antihistamines have been thought of as H_1 receptor antagonists; however, it has recently been demonstrated that they may act as inverse agonists, stabilizing inactive forms of the H_1 and H_2 receptors [43–45]. Whether this is essential or clinically important for antihistamines has not yet been clarified [46]. The second-generation antihistamines terfenadine, astemizole, cetirizine, and loratadine and their efficacy against AR symptoms were investigated in the EEU [47•]. Through these studies, cetirizine and terfenadine continuously ranked higher than loratadine and astemizole in terms of efficacy and onset of action [47•]. This was followed by a study with increased

participant numbers that confirmed that cetirizine significantly reduces symptoms compared to both loratadine and placebo, with an onset of action of 60 min [31]. In the VCC, levocetirizine was compared to loratadine in seasonal AR (challenge to grass pollen) and perennial AR (challenge to house dust mite) [48]. This study demonstrated superiority of levocetirizine in improving seasonal AR symptoms, and a trend towards the same in perennial AR [48]. Levocetirizine was compared to desloratadine in the EEU, revealing that while both were effective, levocetirizine produced a greater improvement in symptom scores during sessions on two consecutive days [49]. Levocetirizine was also examined in the VCC, with consistent results, revealing a longer duration of action than fexofenadine [50]. Fexofenadine was compared to placebo in the EEU and provided clinically important relief with onset of action at 60 min [51]. Cetirizine and fexofenadine were further examined in the EEU, demonstrating a longer duration of action of cetirizine [52, 53•]. Comparative studies continued in the VCC as well, bilastine and cetirizine were found to exhibit longer durations of action, compared to fexofenadine [54•].

Prophylactic experimental designs have also been employed. In the Chiba environmental challenge chamber, participants were exposed to Japanese cedar pollen after a single administration of levocetirizine, levocetirizine for 8 days, or placebo [55•]. Symptoms were lower in both treated groups, compared to placebo, but prophylactic treatment was not superior to single treatment [55•]. This study illustrated an important feature of the fundamental efficacy characteristics of antihistamines. Namely, that there is no added benefit to taking antihistamines continuously, unless exposure to allergen is continuous. Finally, newer antihistamines, with additional pharmacologic properties, have also been examined using CACF models. Rupatadine, a second generation antihistamine and platelet-activating factor antagonist was investigated for prophylactic utility in the VCC [56]. Subjective symptoms and mean secretion weights were significantly lower [56], demonstrating the efficacy of rupatadine, compared to placebo.

Intranasal Antihistamine and/or Steroid Formulations

Intranasal antihistamines, steroids, and combination therapies, and the importance of route of administration, through comparison to oral treatments, have been examined in CACF studies. Recently, the EEU was used to examine azelastine nasal spray vs. orally administered cetirizine, loratadine, or placebo [57]. Azelastine nasal spray significantly reduced nasal symptoms, compared with placebo, and exhibited a faster onset of action compared to oral treatments [57]. An assessment of the onset and duration of action of olopatadine nasal spray was conducted in the EEC by Patel et al. [58]. Olopatadine was significantly more effective than placebo at all time-points starting at 90 min and continuing over 12 h [58]. Azelastine nasal spray was compared to desloratadine tablets in the VCC [59]. While desloratadine significantly improved symptom scores, azelastine nasal spray was superior [59]. Azelastine nasal spray exhibited an onset of action of 15 min, consistent with the onset of action determined in the EEU [59]. Thus, CACF studies have demonstrated consistent results across sites, both in terms of the improvement over systemic treatment, and the onset of action.

A wide variety of intranasal corticosteroids have been approved for treatment of AR, and many have been examined in CACF models. In 1996, the first evaluation of a nasal corticosteroid was performed in the EEU, and revealed a 10 h onset of action of triamcinolone acetonide, which was substantially shorter than expected, as it was previously thought that nasal corticosteroids required several days of administration for efficacy [60••]. A 7 h onset of action was subsequently determined for budesonide in the EEU [61], and a 6 h onset of action for ciclesonide (200mcg) was determined in Mississauga, Ontario [62]. Another assessment of ciclesonide by the same group found an onset of action of 1 h [63], a sizeable difference that is uncharacteristic of a CACF model study.

Another intranasal steroid, fluticasone furoate, was studied at the VCC [64]. Participants were exposed to grass pollen following 8 days of treatment, demonstrating significant reduction in symptoms compared to placebo [64]. Mometasone was compared to azelastine and placebo using a CACF model [12]. Azelastine showed greater reduction in symptoms, with a rapid onset of action starting at 15 min [12]. A subsequent study using 200 mcg mometasone furoate showed a significant difference between ragweed allergic participants who received the treatment and those who were administered the placebo once daily for 8 days [65]. The onset of action was estimated at 6 h, and its duration of action was found to exceed 24 h [65].

CACFs were also used to compare the efficacy of the intranasal anti-histamine olopatadine to mometasone (50 mcg) in ragweed allergic participants [66]. Olopatadine had an earlier onset of action at 30 min compared to 2.5 h [66]. Olopatadine demonstrated a reduction in TNSS for the duration of the study, up to 12 h post administration of the single dose [66]. At Chiba University, cedar pollen allergic individuals were randomized to receive either fexofenadine or mometasone for 7 days to evaluate prophylaxis [67]. Although TNSS in both groups were not different following 3 h of pollen exposure, a difference was present on days 8 to 11 from the start of treatment, suggesting that intranasal steroids had a prolonged anti-inflammatory effect, compared to the antihistamine [67].

A study comparing the solubilized form with the suspended form of budesonide, in combination with azelastine, found all active treatments demonstrated significant decreases

in TNSS, compared to placebo, but the solubilized forms offered greater relief and demonstrated a faster onset of action [68]. Thus, CACF models have been instrumental in demonstrating the efficacy of intranasal steroids, their relative efficacy compared to medications targeting other pathways, determining their onset of action, and exploring characteristics of different formulations.

Decongestants and Combination Therapies

While antihistamines are consistently effective against a range of AR symptoms, they are notably lacking in efficacy against congestion, one of the most bothersome symptoms of AR [69, 70, 71••]. As congestion may be mediated by dilatation of venous capacitance vessels, sympathomimetic agents such as pseudoephedrine are effective decongestants [72–74]. Thus, combination treatments have been developed, including formulations of pseudoephedrine plus antihistamines. The congestion-specific efficacy of an oral formulation of cetirizine/pseudoephedrine was compared to budesonide nasal spray in the VCC [75]. Rhinomanometry demonstrated superior efficacy of the combination therapy for nasal congestion [75]. A further study employing the VCC compared the decongestant properties of phenylephrine and pseudoephedrine [76•]. They found that phenylephrine was not significantly different from placebo in terms of participant-scored nasal congestion, whereas pseudoephedrine was [76•]. At approximately the same time, phenylephrine was investigated in the EEU and compared to loratadine-montelukast and placebo [77]. Similarly, phenylephrine did not significantly reduce symptoms, relative to placebo, while loratadine-montelukast was effective [77]. A further CACF study compared the effect sizes of antihistamine vs. pseudoephedrine alone and in combination, relative to placebo [78•]. Participants underwent four 6 h pollen exposures at the Fraunhofer Institute, with administration of drug after 2 h, in a double-blind, four-way crossover [78•]. Nasal obstruction was significantly lower after treatment with cetirizine/pseudoephedrine, compared to either treatment alone or placebo [78•]. Thus, CACF models played a significant role in establishing the efficacy of combination therapies and contributed to the discussion surrounding the level of efficacy of phenylephrine.

Combinations of antihistamines with sympathomimetic agents have been proven effective against congestion; however, due to side effects such as insomnia and hypertension, they are contraindicated in those with cardiovascular problems [73, 74, 79, 80]. H_3 receptor antagonists as decongestants were suggested as potentially safer alternatives. At the Fraunhofer Institute, dual H_1/H_3 receptor antagonists significantly attenuated nasal symptoms and blockage, relative to placebo, but were not superior to cetirizine [81]. Barchuk et al. investigated a specific H_3 receptor antagonist, compared to placebo, and pseudoephedrine as an active control [82]. Hourly minimal cross-sectional area measurements revealed less of a decrease in nasal patency for the H_3 receptor antagonist versus placebo, which was of borderline significance ($p=0.06$) [82]. The same measure for pseudoephedrine was statistically significant [82]. However, the H_3 receptor antagonist showed superior efficacy compared to pseudoephedrine when the baseline-adjusted area under the curve of participant-scored congestion was used [82]. Recently, a placebo-controlled crossover study of a specific H_3 receptor antagonist with or without fexofenadine was conducted in the EEU and compared to pseudoephedrine/fexofenadine as an active control [80]. Although the combination of H_3 antagonist/fexofenadine significantly reduced TNSS, relative to placebo, with an onset of action of 60 min, the treatment was not superior to pseudoephedrine/fexofenadine [80]. Additionally, an elevated incidence of adverse events was noted, potentially related to the role of H_3 receptors in the central-nervous system [80, 83, 84]. Further development of H_3 receptor antagonists with lesser penetrance into the central nervous system may exhibit a more favorable safety profile, or even increased efficacy against allergic rhinitis symptoms [80].

Antileukotrienes

CACF models have similarly provided key information regarding the efficacy and pharmacodynamics of antileukotrienes [85]. Montelukast was compared to levocetirizine in the EEU with ragweed allergic participants. Although both medications reduced symptoms, levocetirizine was significantly more effective 24 h after the first dose and 4.5 h after the second dose [86•]. A similar study found participants treated with levocetirizine experienced significantly lower symptom scores compared to placebo and montelukast [87]. The onset of action of levocetirizine was estimated at 2.5 h, whereas montelukast could not be evaluated since it did not achieve significant efficacy [87].

Combination therapies involving antileukotrienes have also been evaluated using CACF models. The onset of action of an oral combination of loratadine/montelukast was evaluated in the EEU [88]. This study revealed an onset of action of 75 min against total symptom scores, nasal congestion, and peak nasal inspiratory flow [88]. A follow-up study found loratadine/montelukast was superior to phenylephrine and placebo in reducing nasal congestion, total nasal symptoms, and non-nasal symptoms [77]. Another study at the EEU determined the onset of action of the same dose of loratadine/montelukast to be 75 min [88]. A similar study conducted at the VCC also investigated a combination tablet of loratadine/montelukast [89]. They found the onset of action to be 105 min, and significantly improved nasal congestion symptoms and rhinomanometry compared to placebo

[89]. Thus, CACF models have been instrumental in determining efficacy and pharmacodynamic features of antileukotrienes, and a close agreement has been demonstrated between facilities.

Immunotherapy

Traditional and newer forms of immunotherapy have been tested for efficacy in CACF models. In 1996, the efficacy of two years of traditional ragweed immunotherapy was demonstrated in the EEU, and symptom scores of recipients were significantly reduced compared to those of non-treated controls after 45 min of pollen exposure [90••]. More recently, CACFs have been employed to contribute to the development of novel immunotherapy medications in phase 2 and 3 clinical trials [91]. In one phase 2b study, participants were treated with four weekly subcutaneous injections of short ragweed pollen allergoid [92]. The vaccine was found to significantly improve symptoms and rhinoconjunctivitis-related quality of life, relative to placebo [92]. In a similar study of an intradermally administered synthetic T-cell epitope vaccine for cat allergy, participants were challenged with cat allergen in a CACF prior to treatment, 18–22 weeks from start of study, and again at 50–54 weeks [93]. Efficacy was demonstrated during the 18–22 week and 1 year follow-up exposures [93]. A sub-lingual immunotherapy study conducted at the VCC evaluated a grass pollen tablet, and participants demonstrated a significant improvement of symptom scores, relative to placebo, during pollen challenges beginning at the first month, and maintained to 2 and 4 months [94]. Thus, CACF models have been useful for determining the efficacy, onset and mechanisms of action of new immunotherapy treatments over the past decade.

Basic Science and Novel/Experimental Therapies

Finally, as CACF models provide a sensitive, specific, and reproducible methodology for allergen challenge, they are also useful for the investigation of basic mechanisms of AR and novel therapies. Researchers at the VCC examined circulating blood cell dynamics in an animal model and in human allergic participants exposed to grass pollen in the same study [95]. A significant drop in erythrocyte counts were found both in the murine model and human participants [95]. This represents a novel basic mechanism that may contribute to the development of AR symptoms. However, the authors did not find agreement in the effects on peripheral leukocyte counts in mice and humans [95].

Many novel and experimental therapies that elucidate basic mechanisms of AR have been investigated in CACF models. Researchers employed the VCC to examine the role of the chemoattractant receptor homologous molecule (CRTH2), which mediates activation of Th2 cells, eosinophils and basophils in response to prostaglandin D(2) [96]. A CRTH2 antagonist or placebo was administered twice daily for 8 days, and participants were exposed to grass pollen on the 2nd and 8th days of treatment [96]. A crossover arm demonstrated a significant effect on the 2nd and 8th days, with some persistence of effects despite the 3-week washout period [96]. Scientists at the Fraunhofer Institute conducted a follow-up study with a different CRTH2 antagonist administered orally at three doses (50, 200, and 400 mg twice daily) [97]. Fluticasone propionate nasal spray (200 μg once daily) and oral montelukast (10 mg once daily) were both employed as active controls and all treatments or placebo were administered for 2 weeks prior to orchard grass exposure [97]. Mean TNSS was significantly reduced versus placebo with 200 mg of CRTH2 antagonist, montelukast and fluticasone propionate, relative to placebo [97]. These studies obtained similar results across sites despite the use of different CRTH2 antagonists, and collectively suggest that CRTH2 antagonists may represent novel AR therapies.

Another novel pathway that has recently been explored is the ion channel transient receptor potential vanilloid 1 (TRPV1) [98]. An intranasal formulation of TRPV1 antagonist was examined in the VCC after 8 days of treatment, 8 days of treatment in combination with fluticasone, fluticasone alone, or placebo [98]. A single 4-h allergen exposure was carried out on day 8, revealing no differences in mean TNSS between TRPV1 antagonist alone and placebo, or between TRPV1 antagonist plus fluticasone and fluticasone alone [98]. This study provides an example of how CACF models can help target effective molecules for further development and bring to light cases where despite promising preclinical data, there is a lack of translation to clinical efficacy, before more costly studies are undertaken.

Conclusions

Controlled allergen challenge facilities provide a unique model system, which overcomes certain challenges evident in standard phase 3 efficacy trials by controlling additional study variables. Symptoms generated in CACF studies compare well to the natural season, demonstrate consistent priming characteristics and the ability to phenotype AR participants. Over the past decade, CACF studies have made important contributions to our understanding of the efficacy, onset of action, and other pharmacodynamic characteristics of AR treatments such as antihistamines, antileukotrienes, immunotherapy, and nasal steroids. CACF models are also well suited to basic science research, owing to their sensitive, specific, and reproducible methodology, and will continue to contribute substantially to the medical literature as

newer therapies for the treatment of allergic rhinitis emerge.

Compliance with Ethics Guidelines

Conflict of Interest Anne K. Ellis declares that she is a speaker for Merck and has received grants from Sun Phama and Circassia. Michelle North, Lisa Steacy, Mena Soliman, and Terry Walker declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108:S147–334. *Seminal paper describing the symptoms and pathophysiology of allergic rhinitis and its impact on comorbid asthma*.

2.•• Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM, Krug N, et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. Clin Exp Allergy Rev. 2006;6:31–59. *A consensus paper on CACF models in allergic rhinitis research*.

3.•• Horak F, Jager S. The Vienna challenge chamber (VCC)—a new method for allergen exposition tests. Wien Klin Wocheschr. 1987;99:509–10. *Validation of the Vienna challenge chamber (VCC)*.

4. Pross HF, Day JH, Clark RH, Lees RE. Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J Allergy Clin Immunol. 1987;79:797–810.

5. Day JH, Lees RE, Clark RH, Pattee PL. Respiratory response to formaldehyde and off-gas of urea formaldehyde foam insulation. Can Med Assoc J. 1984;131:1061–5.

6. Ellis AK, North ML, Walker T, Steacy LM. Environmental exposure unit: a sensitive, specific, and reproducible methodology for allergen challenge. Ann Allergy Asthma Immunol. 2013;111:323–8.

7.• Ronborg SM, Mosbech H, Johnsen CR, Poulsen LK. Exposure chamber for allergen challenge. The development and validation of a new concept. Allergy. 1996;51:82–8. *Validation of an allergen exposure facility in Copenhagen, Denmark, for use with house-dust mite allergen*.

8.•• Krug N, Hohlfeld JM, Larbig M, Buckendahl A, Badorrek P, Geldmacher H, et al. Validation of an environmental exposure unit for controlled human inhalation studies with grass pollen in patients with seasonal allergic rhinitis. Clin Exp Allergy. 2003;33:1667–74. *Validation of the Fraunhofer Institute Environmental Challenge Chamber (ECC) in Hannover, Germany.*

9. Berkowitz RB, Woodworth GG, Lutz C, Weiler K, Weiler J, Moss M, et al. Onset of action, efficacy, and safety of fexofenadine 60 mg/pseudoephedrine 120 mg versus placebo in the Atlanta allergen exposure unit. Ann Allergy Asthma Immunol. 2002;89:38–45.

10. Wilken JA, Berkowitz R, Kane R. Decrements in vigilance and cognitive functioning associated with ragweed-induced allergic rhinitis. Ann Allergy Asthma Immunol. 2002;89:372–80.

11. Berkowitz RB, McCafferty F, Lutz C, Bazelmans D, Godfrey P, Meeves S, et al. Onset of action of fexofenadine hydrochloride 60 mg/pseudoephedrine hydrochloride 120 mg in subjects aged 12 years with moderate to severe seasonal allergic rhinitis: a pooled analysis of two single-dose, randomized, double-blind, placebo-controlled allergen exposure unit studies. Clin Ther. 2006;28:1658–69.

12. Patel P, D'Andrea C, Sacks HJ. Onset of action of azelastine nasal spray compared with mometasone nasal spray and placebo in subjects with seasonal allergic rhinitis evaluated in an environmental exposure chamber. Am J Rhinol. 2007;21:499–503.

13. Patel P, Salapatek AM. Pollinex Quattro: a novel and well-tolerated, ultra short-course allergy vaccine. Expert Rev Vaccines. 2006;5:617–29.

14. Ramirez DA JR, Andrews CP. Uniperus asheii (mountain cedar) pollen utilized as an antigen in the biogenics chamber: comparison of natural and controlled exposures. J Allergy Clin Immunol. 2011;127:AB19.

15. Jacobs RL, Ramirez DA, Andrews CP. Validation of the biogenics research chamber for Juniperus ashei (mountain cedar) pollen. Ann Allergy Asthma Immunol. 2011;107:133–8.

16. Crampton H.J., Gomes, P. A Pilot Study Evaluating the Signs and Symptoms of Seasonal Allergic Rhinitis and Conjunctivitis Following Allergen Exposure in the Allergen BioCube. ClinicalTrials.gov 2009; identifier: NCT00985075.

17.• Hamasaki S, Okamoto Y, Yonekura S, Okuma Y, Sakurai T, Iinuma T, et al. Characteristics of the Chiba environmental challenge chamber. Allergol Int. 2014;63:41–50. *Validation of the Chiba environmental challenge chamber in Japan*.

18. Ito K, Terada T, Yuki A, Ichihara T, Hyo S, Kawata R, et al. Preliminary study of a challenge test to the patients with Japanese cedar pollinosis using an environmental exposure unit. Auris Nasus Larynx. 2010;37:694–9.

19. Yang WH, Yang J, Perrins R, Kelly S, Karsh J. Computer-aided design of an allergen challenge theatre. J Allergy Clin Immunol. 2014;133:AB187.

20. Muradil M, Okamoto Y, Yonekura S, Chazono H, Hisamitsu M, Horiguchi S, et al. Reevaluation of pollen quantitation by an automatic pollen counter. Allergy Asthma Proc. 2010;31:422–7.

21. Chervinsky P, Philip G, Malice MP, Bardelas J, Nayak A, Marchal JL, et al. Montelukast for treating fall allergic rhinitis: effect of pollen exposure in 3 studies. Ann Allergy Asthma Immunol. 2004;92:367–73.

22. Akerlund A, Andersson M, Leflein J, Lildholdt T, Mygind N. Clinical trial design, nasal allergen challenge models, and considerations of relevance to pediatrics, nasal polyposis, and different classes of medication. J Allergy Clin Immunol. 2005;115:S460–82.

23. Day JH, Ellis AK, Rafeiro E, Ratz JD, Briscoe MP. Experimental models for the evaluation of treatment of allergic rhinitis. Ann Allergy Asthma Immunol. 2006;96:263–77. *quiz 277–8, 315*.

24. U.S. Food and Drug Administration Center for Biologics Evaluation and Research: Allergenic Products Advisory Committee. Transcript, Capital Reporting Company 2011. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/AllergenicProductsAdvisoryCommittee/UCM258587.pdf Accessed 10 Dec 2014.

25. Bernstein JA. Correlation between a pollen challenge chamber and a natural allergen exposure study design for eliciting ocular and nasal symptoms: early evidence supporting a paradigm shift in drug investigation? J Allergy Clin Immunol. 2012;130:128–9.

26. Devillier P, Le Gall M, Horak F. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. Allergy. 2011;66:163–9.
27. Committee for Medicinal Products for Human Use (CHMP). Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases. Doc. Ref. CHMP/EWP/18504/2006. 2008. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003605.pdf Accessed 10 Dec 2014.
28. Horak F, Stubner UP, Zieglmayer R, Harris AG. Effect of desloratadine versus placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. J Allergy Clin Immunol. 2002;109:956–61.
29. Horak F, Stubner P, Zieglmeyer R, Harris AG. Comparison of the effects of desloratadine 5-mg daily and placebo on nasal airflow and seasonal allergic rhinitis symptoms induced by grass pollen exposure. Allergy. 2003;58:481–5.
30. Day JH, Briscoe M, Rafeiro E, Chapman D, Kramer B. Comparative onset of action and symptom relief with cetirizine, loratadine, or placebo in an environmental exposure unit in subjects with seasonal allergic rhinitis: confirmation of a test system. Ann Allergy Asthma Immunol. 2001;87:474–81.
31. Day JH, Briscoe M, Widlitz MD. Cetirizine, loratadine, or placebo in subjects with seasonal allergic rhinitis: effects after controlled ragweed pollen challenge in an environmental exposure unit. J Allergy Clin Immunol. 1998;101:638–45.
32.•• Connell JT. Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. J Allergy. 1969;43:33–44. *Seminal work on the priming effect*.
33. Yuki A, Terada T, Ichihara T, Fujii K, Hyo S, Kawata R, et al. Evaluating the effects of testing period on pollinosis symptoms using an allergen challenge chamber. Allergol Int. 2011;60:533–9.
34.• Ellis AK, Ratz JD, Day AG, Day JH. Factors that affect the allergic rhinitis response to ragweed allergen exposure. Ann Allergy Asthma Immunol. 2010;104:293–8. *Important paper that demonstrated common individual participant factors that affect symptom response in CACF studies*.
35. Jacobs, R.L., Andrews, C.P., Ramirez, D.A., Rather, C.G., Harper, N., Jimenez, F, et al. Symptom dynamics during repeated serial allergen challenge chamber exposures to house dust mite. J Allergy Clin Immunol. 2014. in press.
36. Ellis AK, Rafeiro E, Day JH. Quality of life indices may be predictive of placebo and medication response to treatment for allergic rhinitis. Ann Allergy Asthma Immunol. 2001;86:393–6.
37.• Ellis AK, Day JH, Lundie MJ. Impact on quality of life during an allergen challenge research trial. Ann Allergy Asthma Immunol. 1999;83:33–9. *Work that demonstrated quality of life effects that result from participation in CACF trials*.
38.•• Badorrek P, Dick M, Hecker H, Schaumann F, Sousa AR, Murdoch R, et al. Anti-allergic drug testing in an environmental challenge chamber is suitable both in and out of the relevant pollen season. Ann Allergy Asthma Immunol. 2011;106:336–41. *Important paper that demonstrated CACF studies may be conducted both in and out of season*.
39. Hohlfeld JM, Holland-Letz T, Larbig M, Lavae-Mokhtari M, Wierenga E, Kapsenberg M, et al. Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber compared with natural conditions. Clin Exp Allergy. 2010;40:998–1006.
40.• Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA, et al. Responses to ragweed pollen in a pollen challenge chamber versus seasonal exposure identify allergic rhinoconjunctivitis endotypes. J Allergy Clin Immunol. 2012;130:122–7 e8. *Demonstration of concordance between symptoms generated in a CACF model compared to the natural season*.
41. Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA, et al. Effect of confounding cofactors on responses to pollens during natural season versus pollen challenge chamber exposure. J Allergy Clin Immunol. 2014;133:1340–6.
42. Soliman, M., Ellis, A.K. Phenotyping Allergic Rhinitis Responses using the Environmental Exposure Unit (EEU). Ann Allergy Asthma Immunol. 2015. in press.
43. Smit MJ, Timmerman H, Alewijnse AE, Leurs R. From histamine H2 receptor regulation to reclassification of H2 antagonists; inverse agonism as the basis for H2 receptor upregulation. Receptors Channels. 1998;5:99–102.
44. Bakker RA, Wieland K, Timmerman H, Leurs R. Constitutive activity of the histamine H(1) receptor reveals inverse agonism of histamine H(1) receptor antagonists. Eur J Pharmacol. 2000;387:R5–7.
45. Wu RL, Anthes JC, Kreutner W, Harris AG, West Jr RE. Desloratadine inhibits constitutive and histamine-stimulated nuclear factor-kappaB activity consistent with inverse agonism at the histamine H1 receptor. Int Arch Allergy Immunol. 2004;135:313–8.
46. Monczor F, Fernandez N, Fitzsimons CP, Shayo C, Davio C. Antihistaminergics and inverse agonism: potential therapeutic applications. Eur J Pharmacol. 2013;715:26–32.
47.• Day JH, Briscoe MP, Clark RH, Ellis AK, Gervais P. Onset of action and efficacy of terfenadine, astemizole, cetirizine, and loratadine for the relief of symptoms of allergic rhinitis. Ann Allergy Asthma Immunol. 1997;79:163–72. *Important investigation of second-generation antihistamines using a CACF model*.
48. Stubner P, Zieglmayer R, Horak F. A direct comparison of the efficacy of antihistamines in SAR and PAR: randomised, placebo-controlled studies with levocetirizine and loratadine using an environmental exposure unit - the Vienna Challenge Chamber (VCC). Curr Med Res Opin. 2004;20:891–902.
49. Day JH, Briscoe MP, Rafeiro E, Ratz JD. Comparative clinical efficacy, onset and duration of action of levocetirizine and desloratadine for symptoms of seasonal allergic rhinitis in subjects evaluated in the Environmental Exposure Unit (EEU). Int J Clin Pract. 2004;58:109–18.
50. Horak F, Zieglmayer PU, Zieglmayer R, Kavina A, Lemell P. Levocetirizine has a longer duration of action on improving total nasal symptoms score than fexofenadine after single administration. Br J Clin Pharmacol. 2005;60:24–31.
51. Day JH, Briscoe MP, Welsh A, Smith JN, Clark A, Ellis AK, et al. Onset of action, efficacy, and safety of a single dose of fexofenadine hydrochloride for ragweed allergy using an environmental exposure unit. Ann Allergy Asthma Immunol. 1997;79:533–40.
52. Day JH, Briscoe MP, Rafeiro E, Ratz JD, Ellis AK, Frankish CW, et al. Comparative efficacy of cetirizine and fexofenadine for seasonal allergic rhinitis, 5–12 hours postdose, in the environmental exposure unit. Allergy Asthma Proc. 2005;26:275–82.
53.• Day JH, Briscoe MP, Rafeiro E, Hewlett Jr D, Chapman D, Kramer B. Randomized double-blind comparison of cetirizine and fexofenadine after pollen challenge in the Environmental Exposure Unit: duration of effect in subjects with seasonal allergic rhinitis. Allergy Asthma Proc. 2004;25:59–68. *Demonstration of a longer duration of action for cetirizine compared to fexofenadine*.
54.• Horak F, Zieglmayer P, Zieglmayer R, Lemell P. The effects of bilastine compared with cetirizine, fexofenadine, and placebo on allergen-induced nasal and ocular symptoms in patients exposed to aeroallergen in the Vienna Challenge Chamber. Inflamm Res. 2010;59:391–8. *Demonstration of longer durations of action for bilastine and cetirizine compared to fexofenadine*.
55.• Yonekura S, Okamoto Y, Yamamoto H, Sakurai T, Iinuma T, Sakurai D, et al. Randomized double-blind study of prophylactic treatment with an antihistamine for seasonal allergic rhinitis. Int Arch Allergy Immunol. 2013;162:71–8. *Demonstration that*

prophylactic treatment with antihistamine is not superior to single treatment.

56. Stuebner P, Horak F, Zieglmayer R, Arnaiz E, Leuratti C, Perez I, et al. Effects of rupatadine vs placebo on allergen-induced symptoms in patients exposed to aeroallergens in the Vienna Challenge Chamber. Ann Allergy Asthma Immunol. 2006;96:37–44.
57. Ellis AK, Zhu Y, Steacy LM, Walker T, Day JH. A four-way, double-blind, randomized, placebo controlled study to determine the efficacy and speed of azelastine nasal spray, versus loratadine, and cetirizine in adult subjects with allergen-induced seasonal allergic rhinitis. Allergy Asthma Clin Immunol. 2013;9:16.
58. Patel P, Roland PS, Marple BF, Benninger PJ, Margalias H, Brubaker M, et al. An assessment of the onset and duration of action of olopatadine nasal spray. Otolaryngol Head Neck Surg. 2007;137:918–24.
59. Horak F, Zieglmayer UP, Zieglmayer R, Kavina A, Marschall K, Munzel U, et al. Azelastine nasal spray and desloratadine tablets in pollen-induced seasonal allergic rhinitis: a pharmacodynamic study of onset of action and efficacy. Curr Med Res Opin. 2006;22:151–7.
60.•• Day JH, Buckeridge DL, Clark RH, Briscoe MP, Phillips R. A randomized, double-blind, placebo-controlled, controlled antigen delivery study of the onset of action of aerosolized triamcinolone acetonide nasal spray in subjects with ragweed-induced allergic rhinitis. J Allergy Clin Immunol. 1996;97:1050–7. *The first evaluation of a nasal corticosteroid in a CACF model that revealed an earlier onset of action than was anticipated*.
61. Day JH, Briscoe MP, Rafeiro E, Ellis AK, Pettersson E, Akerlund A. Onset of action of intranasal budesonide (Rhinocort aqua) in seasonal allergic rhinitis studied in a controlled exposure model. J Allergy Clin Immunol. 2000;105:489–94.
62. Couroux P, Kunjibettu S, Hall N, Wingertzahn MA. Onset of action of ciclesonide once daily in the treatment of seasonal allergic rhinitis. Ann Allergy Asthma Immunol. 2009;102:62–8.
63. Patel P, Patel D, Kunjibettu S, Hall N, Wingertzahn MA. Onset of action of ciclesonide once daily in the treatment of seasonal allergic rhinitis. Ear Nose Throat J. 2008;87:340–53.
64. Zieglmayer P, Zieglmayer R, Bareille P, Rousell V, Salmon E, Horak F. Fluticasone furoate versus placebo in symptoms of grass-pollen allergic rhinitis induced by exposure in the Vienna challenge chamber. Curr Med Res Opin. 2008;24:1833–40.
65. Salapatek AM, Patel P, Gopalan G, Varghese ST. Mometasone furoate nasal spray provides early, continuing relief of nasal congestion and improves nasal patency in allergic patients. Am J Rhinol Allergy. 2010;24:433–8.
66. Patel D, Garadi R, Brubaker M, Conroy JP, Kaji Y, Crenshaw K, et al. Onset and duration of action of nasal sprays in seasonal allergic rhinitis patients: olopatadine hydrochloride versus mometasone furoate monohydrate. Allergy Asthma Proc. 2007;28:592–9.
67. Yamamoto H, Yonekura S, Sakurai D, Katada K, Inamine A, Hanazawa T, et al. Comparison of nasal steroid with antihistamine in prophylactic treatment against pollinosis using an environmental challenge chamber. Allergy Asthma Proc. 2012;33:397–403.
68. Salapatek AM, Lee J, Patel D, D'Angelo P, Liu J, Zimmerer Jr RO, et al. Solubilized nasal steroid (CDX-947) when combined in the same solution nasal spray with an antihistamine (CDX-313) provides improved, fast-acting symptom relief in patients with allergic rhinitis. Allergy Asthma Proc. 2011;32:221–9.
69. Simons FE. Comparative pharmacology of H1 antihistamines: clinical relevance. Am J Med. 2002;113(Suppl 9A):38S–46.
70. del Cuvillo A, Mullol J, Bartra J, Davila I, Jauregui I, Montoro J, et al. Comparative pharmacology of the H1 antihistamines. J Investig Allergol Clin Immunol. 2006;16 Suppl 1:3–12.
71.•• Nathan RA, Meltzer EO, Derebery J, Campbell UB, Stang PE, Corrao MA, et al. The prevalence of nasal symptoms attributed to allergies in the United States: findings from the burden of rhinitis in an America survey. Allergy Asthma Proc. 2008;29:600–8. *Important work on the prevalence and impact of AR in the USA*.
72. Djukanovic R, Wilson SJ, Howarth PH. Pathology of rhinitis and bronchial asthma. Clin Exp Allergy. 1996;26 Suppl 3:44–51.
73. Chervinsky P, Nayak A, Rooklin A, Danzig M. Efficacy and safety of desloratadine/pseudoephedrine tablet, 2.5/120 mg two times a day, versus individual components in the treatment of patients with seasonal allergic rhinitis. Allergy Asthma Proc. 2005;26:391–6.
74. Moinuddin R, de Tineo M, Maleckar B, Naclerio RM, Baroody FM. Comparison of the combinations of fexofenadine-pseudoephedrine and loratadine-montelukast in the treatment of seasonal allergic rhinitis. Ann Allergy Asthma Immunol. 2004;92:73–9.
75. Zieglmayer UP, Horak F, Toth J, Marks B, Berger UE, Burtin B. Efficacy and safety of an oral formulation of cetirizine and prolonged-release pseudoephedrine versus budesonide nasal spray in the management of nasal congestion in allergic rhinitis. Treat Respir Med. 2005;4:283–7.
76.• Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Yao R, Staudinger H, et al. A placebo-controlled study of the nasal decongestant effect of phenylephrine and pseudoephedrine in the Vienna Challenge Chamber. Ann Allergy Asthma Immunol. 2009;102:116–20. *Important work on the relative efficacy of pseudoephedrine and phenylephrine*.
77. Day JH, Briscoe MP, Ratz JD, Danzig M, Yao R. Efficacy of loratadine-montelukast on nasal congestion in patients with seasonal allergic rhinitis in an environmental exposure unit. Ann Allergy Asthma Immunol. 2009;102:328–38.
78.• Badorrek P, Dick M, Schauerte A, Hecker H, Murdoch R, Luettig B, et al. A combination of cetirizine and pseudoephedrine has therapeutic benefits when compared to single drug treatment in allergic rhinitis. Int J Clin Pharmacol Ther. 2009;47:71–7. *Important comparison of the efficacy of antihistamine vs. pseudoephedrine alone and in combination*.
79. Empey DW, Young GA, Letley E, John GC, Smith P, McDonnell KA, et al. Dose–response study of the nasal decongestant and cardiovascular effects of pseudoephedrine. Br J Clin Pharmacol. 1980;9:351–8.
80. North ML, Walker T, Steacy LM, Hobsbawn BG, Allan RJ, Hackman F, et al. Double blind randomized crossover trial of PF-03654764 + fexofenadine in the environmental exposure unit (EEU). Allergy Asthma Clin Immunol. 2014;10:A68.
81. Daley-Yates P, Ambery C, Sweeney L, Watson J, Oliver A, McQuade B. The efficacy and tolerability of two novel H(1)/H(3) receptor antagonists in seasonal allergic rhinitis. Int Arch Allergy Immunol. 2012;158:84–98.
82. Barchuk WT, Salapatek AM, Ge T, D'Angelo P, Liu X. A proof-of-concept study of the effect of a novel H3-receptor antagonist in allergen-induced nasal congestion. J Allergy Clin Immunol. 2013;132:838–46. e1-6.
83. Wager TT, Pettersen BA, Schmidt AW, Spracklin DK, Mente S, Butler TW, et al. Discovery of two clinical histamine H(3) receptor antagonists: trans-N-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidinylmethyl)phenyl]cyclobutanecarbox amide (PF-03654746) and trans-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl]-N-(2-methylpropyl)cyclobutanecarboxamide (PF-03654764). J Med Chem. 2011;54:7602–20.
84. Weisler RH, Pandina GJ, Daly EJ, Cooper K, Gassmann-Mayer C. Randomized clinical study of a histamine H3 receptor antagonist for the treatment of adults with attention-deficit hyperactivity disorder. CNS Drugs. 2012;26:421–34.
85. Small P, Kim H. Allergic rhinitis. Allergy Asthma Clin Immunol. 2011;7 Suppl 1:S3.
86.• Day JH, Briscoe MP, Ratz JD. Efficacy of levocetirizine compared with montelukast in subjects with ragweed-induced seasonal allergic rhinitis in the Environmental Exposure Unit. Allergy Asthma

Proc. 2008;29:304–12. *Comparison of the antileukotriene montelukast to the antihistamine levocetirizine.*

87. Patel P, Patel D. Efficacy comparison of levocetirizine vs montelukast in ragweed sensitized patients. Ann Allergy Asthma Immunol. 2008;101:287–94.
88. Day JH, Briscoe MP, Ratz JD, Ellis AK, Yao R, Danzig M. Onset of action of loratadine/montelukast in seasonal allergic rhinitis subjects exposed to ragweed pollen in the environmental exposure unit. Allergy Asthma Proc. 2009;30:270–6.
89. Horak F, Zieglmayer P, Zieglmayer R, Lemell P. Onset of action of loratadine/montelukast in seasonal allergic rhinitis patients exposed to grass pollen. Arzneimittelforschung. 2010;60:249–55.
90.•• Donovan JP, Buckeridge DL, Briscoe MP, Clark RH, Day JH. Efficacy of immunotherapy to ragweed antigen tested by controlled antigen exposure. Ann Allergy Asthma Immunol. 1996;77:74–80. *Important evaluation of the efficacy of standard immunotherapy.*
91. Ellis AK. Environmental exposure units for specific immunotherapy trials. Arb Paul Ehrlich Inst Bundesinstitut Impfstoffe Biomed Arzneim Langen Hess. 2013;97:91–4.
92. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. J Allergy Clin Immunol. 2014;133:121–9 e1-2.
93. Patel D, Couroux P, Hickey P, Salapatek AM, Laidler P, Larche M, et al. el d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. J Allergy Clin Immunol. 2013;131:103–9 e1-7.
94. Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy Clin Immunol. 2009;124:471–7, 477 e1.
95. Jordakieva G, Wallmann J, Schmutz R, Lemell P, Wegmann M, Nittke T, et al. Peripheral erythrocytes decrease upon specific respiratory challenge with grass pollen allergen in sensitized mice and in human subjects. PLoS One. 2014;9:e86701.
96. Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Collins LP, Hunter MG, et al. The CRTH2 antagonist OC000459 reduces nasal and ocular symptoms in allergic subjects exposed to grass pollen, a randomised, placebo-controlled, double-blind trial. Allergy. 2012;67:1572–9.
97. Krug N, Gupta A, Badorrek P, Koenen R, Mueller M, Pivovarova A, et al. Efficacy of the oral chemoattractant receptor homologous molecule on TH2 cells antagonist BI 671800 in patients with seasonal allergic rhinitis. J Allergy Clin Immunol. 2014;133:414–9.
98. Bareille P, Murdoch RD, Denyer J, Bentley J, Smart K, Yarnall K, et al. The effects of a TRPV1 antagonist, SB-705498, in the treatment of seasonal allergic rhinitis. Int J Clin Pharmacol Ther. 2013;51:576–84.