



## Review

## Advances in rhinitis—models and mechanisms

Anne K. Ellis, MD, MSc, FRCPC <sup>\*,†</sup>; Mark W. Tenn, BHSc <sup>\*,†</sup><sup>\*</sup> Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada<sup>†</sup> Departments of Medicine and Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

## ARTICLE INFO

## Article history:

Received for publication September 5, 2017.

Received in revised form September 30, 2017.

Accepted for publication October 4, 2017.

## ABSTRACT

**Objective:** To summarize studies highlighting recent advances in rhinitis-related research in the past 2 years.**Data Sources:** Original research articles were procured and examined from the Rhinitis and Upper Airway Disease section of the 2015 to 2017 *Annals of Allergy, Asthma & Immunology* issues. Additional original research articles were identified from PubMed and Google Scholar using the following search terms: *allergic rhinitis*, *rhinitis*, *chronic rhinosinusitis*, *environmental exposure unit*, and *nasal allergen challenge*. Only research articles published in the past 2 years were procured.**Study Selections:** Articles conducting research in allergic rhinitis (AR) or chronic rhinosinusitis or using controlled allergen challenge facilities or the nasal allergen challenge model were selected.**Results:** Studies in the past 2 years have focused on using skin prick tests and early-life phenotyping to predict AR development in children. They also have elucidated the role of a subset of CD4<sup>+</sup> T cells, basophils, and mast cells in non-eosinophilic chronic rhinosinusitis with nasal polyps, a relatively new chronic rhinosinusitis subtype in the Asian population. Several advances have been made in understanding the role of several cytokines and peripheral cell mitochondrial function in AR using controlled allergen challenge facilities and direct nasal allergen challenges.**Conclusion:** Findings from the recent literature highlight the utility of early-life predictors of AR in possibly targeting high-risk groups for prophylactic interventions. Studies also emphasize the use of controlled allergen challenge facilities and the nasal allergen challenge model as robust experimental models to study AR pathogenesis.

© 2017 American College of Allergy, Asthma &amp; Immunology. Published by Elsevier Inc. All rights reserved.

## Introduction

During the past 2 years, the *Annals* has published several key studies that have advanced our understanding of allergic rhinitis (AR), chronic rhinosinusitis (CRS), and the experimental models used to study them. This article summarizes the main findings and highlights those with clinical significance.

## Recent Advances in AR

Allergic rhinitis is an upper airway inflammatory disorder characterized by immunoglobulin E (IgE)-mediated inflammation of the

nasal mucosa. In recent years, the prevalence of AR has rapidly increased, with the disease affecting 10% to 40% of the global population, and is one of the most common medical conditions in children.<sup>1,2</sup> Current research has focused on identifying early-life predictors of AR development.

Codispoti et al<sup>3</sup> evaluated the predictive value of skin prick test wheal areas during the first 3 years of life for the development of allergic eosinophilic rhinitis at 4 years of age using the Cincinnati Childhood Allergy and Air Pollution Study cohort. This prospective birth cohort followed high-risk infants born to aeroallergen-sensitized and symptomatic parents since birth. Physical examinations, parental interviews, and skin prick tests to common aeroallergens were conducted annually, and nasal epithelial smears were collected at 4 years of age to determine allergic eosinophilic rhinitis. Codispoti et al found a significant association of 3-year skin prick test wheal areas to *Penicillium* species (adjusted odds ratio [aOR] 1.18) and maple (aOR 1.07) and a borderline association for elm (aOR 1.06) with the development of allergic eosinophilic rhinitis at 4 years of age.<sup>3</sup> Another study sought to identify rhinitis phenotypes for the prognosis of asthma development in children enrolled in the Children's Health and Environmental Research cohort with current rhinitis. This prospective cohort conducted follow-up tests and surveys at the time of enrollment and at 2 and 4 years

**Reprints:** Anne K. Ellis, MD, MSc, FRCPC, Department of Medicine, Division of Allergy and Immunology with a cross-appointment to the Department of Biomedical and Molecular Sciences, Kingston General Hospital, Watkins D1, 76 Stuart Street, Kingston, ON K7L 2V7, Canada; E-mail: [ellis.anne@gmail.com](mailto:ellis.anne@gmail.com).

**Disclosures:** Dr Ellis has participated in advisory boards for ALK Abello, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Merck, and Novartis and has been a speaker for Aralez, AstraZeneca, Boehringer Ingelheim, Meda, Merck, Novartis, PediaPharm, Pfizer, and Takeda. Her institution has received research grants from Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sanofi, Sun Pharma, Merck, Novartis, and Pfizer. Dr Ellis also is a consultant to Bayer Inc. Mr Tenn has nothing to disclose.

of age. Using latent cluster analysis, Lee et al<sup>4</sup> identified 4 rhinitis phenotypes in this population: cluster 1 (24.8%) with a very small atopic burden (presence of specific IgE to aeroallergens) and low socioeconomic status; cluster 2 (36.3%) with high atopic burden and normal lung function; cluster 3 (21.9%) with high atopic burden and impaired lung function; and cluster 4 (17.0%) with small atopic burden and high socioeconomic status. Of the phenotypes, children belonging to cluster 3 demonstrated the largest percentage of blood eosinophils and total serum IgE levels at enrollment and the highest incidence of asthma and bronchial hyperresponsiveness cases at follow-up.<sup>4</sup> Overall, the studies highlight the utility of skin testing for specific allergens and early-life phenotyping in identifying children at risk of developing asthma.

Gelardi et al<sup>5</sup> detailed the use of nasal cytology as a low-cost and effective diagnostic procedure for assessing AR. By performing a nasal scraping, May-Grunwald-Giemsa staining, and optical microscopy, it is possible to identify inflammatory cells and predominant cell types. This leads to a fast and accurate diagnosis of non-AR, AR, and viral infections. A detailed assessment of disease, which can be gained through the use of nasal cytology, leads to an increase in knowledge and efficiency in research, diagnosis, and therapy.

### Recent Advances in CRS

Chronic rhinosinusitis is an inflammatory disorder of the nasal and sinus passages and is defined as having active symptoms (eg, nasal obstruction, postnasal drip, facial pressure) lasting at least 12 weeks without complete resolution.<sup>6</sup> CRS affects 1% to 9% of the general population and exhibits 2 traditional clinical phenotypes, CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). CRSwNP is characterized by T-helper cell type 2-skewed inflammation, whereas CRSsNP exhibits a T-helper cell type 1-mediated inflammatory response.<sup>6</sup> However, these classifications are continually evolving because of the increasing complexity and appearance of different CRS endotypes defined by unique pathophysiologic mechanisms.

Several studies have described a new subtype of CRSwNP in East Asia, characterized by T-helper cell type 1-mediated non-eosinophilic inflammation in nasal polyps (NPs) and a different comorbidity rate of asthma compared with classic CRSwNP.<sup>7-9</sup> Denoted as non-eosinophilic CRS (NECRS), Iinuma et al<sup>10</sup> characterized tissue CD4<sup>+</sup> T cells collected from NP samples from patients with CRSwNP classified as having NECRS or eosinophilic CRS (E CRS; akin to classic CRSwNP). They noted increased levels of interleukin (IL)-25 from NPs and increased IL-5, IL-9, and IL-17RB mRNA expression in NP CD4<sup>+</sup> T cells from patients with E CRS vs patients with NECRS. Increased IL-5 and IL-9 production from NP mononuclear cells after IL-25 and T-cell receptor stimulation also was observed only from patients with E CRS and not those with NECRS.<sup>10</sup> In another study assessing the pathologic differences between E CRS and NECRS, Kagoya et al<sup>11</sup> reported a significant increase in basophils in NPs from patients with E CRS vs patients with NECRS. Furthermore, NP basophil counts were positively correlated with the Lund-Mackey composite score (self-reported assessment of CRSwNP symptom severity) only in patients with E CRS.<sup>11</sup> A study published in this year's *Annals* evaluated the role and phenotypes of NP mast cells in patients with E CRS and those with NECRS. Baba et al<sup>12</sup> found a significant increase in NP mast cells in patients with E CRS and those with NECRS compared with controls, with mast cell infiltration primarily localized to the epithelium and glands of E CRS polyps and the submucosa of NECRS polyps. Infiltrating mast cells to these regions were primarily positive for tryptase and doubly positive for tryptase and chymase in E CRS and NECRS polyps, respectively. E CRS polyps also exhibited a significant increase in IgE<sup>+</sup> mast cells, with few detected in NECRS polyps.<sup>12</sup> Together, these studies illustrate the pathologic differences between the 2 subtypes of CRSwNP; sub-

populations of CD4<sup>+</sup> T cells, basophils, and IgE<sup>+</sup> mast cells could contribute to the eosinophilic infiltration and overall pathogenesis of E CRS.

Chronic rhinosinusitis is often accompanied by comorbid asthma in children and adults. In 2 recent studies, 47% of children had evidence of rhinosinusitis after endoscopic examination and 50% of adults with CRS had concomitant asthma.<sup>13,14</sup> Medical and surgical management of CRS have been shown to alleviate asthma symptoms and improve lung function.<sup>15-17</sup> Owing to the association between the 2 diseases, Anfuso et al<sup>18</sup> evaluated the impact of comorbid asthma on the upper airway inflammatory response in children with CRS refractory to medical management. Sinus and adenoid tissue samples were collected from children with CRS with and without asthma and analyzed for mucosal expression of 40 inflammatory cytokines. Inflammatory profiles in sinus and adenoid tissues were more severe in children with CRS and asthma compared with children with CRS without asthma. Significant increases were observed for sinus levels of tumor necrosis factor- $\beta$  and adenoid levels of platelet-derived growth factor-AA, eotaxin, epidermal growth factor, growth-related oncogene, and fibroblast growth factor-2.<sup>18</sup> In a cross-sectional study involving adults with CRS and asthma, Phillips et al<sup>19</sup> evaluated the association between CRS symptom severity and asthma control. Severity of CRS symptoms and degree of asthma control were measured using the 22-item Sinonasal Outcome Test and the Asthma Control Test, respectively. After controlling for age, sex, presence of nasal polyps, at least 1 aeroallergen sensitivity, use of asthma controller inhaler, and smoking status, Phillips et al observed a negative association between CRS symptom severity and degree of asthma control ( $P = .02$ ). CRS severity also was significantly associated with poor asthma control (defined as Asthma Control Test score  $\leq 19$ ; aOR 1.06).<sup>19</sup>

### Controlled Allergen Challenge Facilities

Controlled allergen challenge facilities (CACFs) are clinically validated experimental models of AR that have been used to evaluate new therapeutics for AR.<sup>20,21</sup> They allow large groups of participants to be exposed to a controlled quantity of aeroallergen under regulated conditions. Confounding factors such as temperature, humidity, and allergen concentration (present in traditional natural exposure studies) can be controlled for, creating a standardized environment within the facility.<sup>20,22</sup> Recent studies published in the *Annals* have used CACFs to phenotype individuals with AR and study AR pathophysiology.

Using the Environmental Exposure Unit, Soliman and Ellis<sup>23</sup> phenotyped participants with ragweed-induced AR based on late-onset nasal symptoms after a 3-hour ragweed pollen challenge in the unit. Participants recorded their total nasal symptom scores (TNSS; composite of nasal congestion, nasal itching, sneezing, and rhinorrhea) and peak nasal inspiratory flow (PNIF) at baseline, 30-minute intervals for the first 3 hours, and hourly until 12 hours after the challenge. Soliman and Ellis identified 3 AR phenotypes based on their pattern of late-onset TNSS (symptoms at hours 6-12) after an initial peak in TNSS at 3 hours. These 3 phenotypes were (1) early-phase responders (EPRs) who demonstrated at least a 50% decrease in TNSS by hours 6 to 7 compared with their peak at 3 hours and returned to baseline by hour 12, (2) protracted EPRs who did not exhibit at least a 50% decrease in TNSS by hour 6 to 7 compared with their peak at 3 hours and did not return to baseline by hour 12, and (3) dual-phase responders who demonstrated at least a 50% decrease in TNSS at hour 6 to 7 compared with their peak at 3 hours, maintained at least 2 hours of decreased symptom severity, followed by a sustained increase in symptom severity by hour 12. Using these criteria, 14 participants were EPRs, 21 were protracted EPRs, and 8 were dual-phase responders. Significant differences in late-onset TNSS profiles also were observed among all 3 phenotypes.<sup>23</sup>

Jacobs et al<sup>24</sup> reported similar phenotypic findings in a population of individuals with ragweed-induced allergic rhinoconjunctivitis using late-onset ocular redness instead of TNSS. Subjects allergic to ragweed underwent 3 3-hour ragweed exposures in an allergen challenge chamber followed by a 7-hour observation period. Ocular redness scores recorded during this period were used to classify individuals as EPRs, protracted EPRs, and dual-phase responders using similar criteria as used by Soliman and Ellis.<sup>23</sup> Similarly, significant differences in ocular redness scores among phenotypes were observed.<sup>24</sup> These 2 studies highlight distinct responsive phenotypes in patients with AR after controlled allergen challenges. Akin to the heterogeneity seen in asthma, AR phenotypes could represent an important consideration during trial enrollment because future interventions might be more suitable for one phenotype over the others.

With their ability to recapitulate the symptoms and inflammatory processes associated with AR, CACFs have been widely used to evaluate local nasal inflammatory responses after controlled allergen exposure. Okuma et al<sup>25</sup> sought to characterize the symptoms and mediators involved in late-phase responses in participants with Japanese cedar pollen-induced AR after 3 hours of continuous allergen exposure in an environmental challenge chamber. TNSS (sneezing, rhinorrhea, nasal congestion) was recorded and nasal secretions were collected during the challenge and 6 hours after exiting the chamber to monitor late-phase inflammation. Okuma et al observed a significant increase in TNSS during the challenge, which peaked at 6 hours after initial pollen exposure. Concentrations of IL-5, IL-13, IL-31, IL-33, histamine, and substance P were significantly increased in nasal secretions after challenge and remained increased after leaving the chamber. Notably, this study did not identify different phenotypic late-phase response groups. Levels of IL-31, histamine, and substance P at 9 hours positively correlated with the total number of sneezing and nose blowing after exiting the chamber, suggesting that these mediators might be involved in prolonging nasal symptoms well after allergen exposure.<sup>25</sup> Another study conducted by Badorrek et al<sup>26</sup> evaluated the specificity and reproducibility of nasal biomarkers after pollen exposure in an allergen challenge chamber. Participants with grass pollen-induced AR underwent 2 4-hour exposures to grass pollen in the facility with a 21-day washout period between exposure periods. Nasal lavage and secretions were collected to assess cell composition and mediators after pollen exposure. Badorrek et al observed significant increases in nasal eosinophils, IL-5, IL-6, IL-13, and macrophage inflammatory protein-1 $\beta$  in participants with AR that were absent in healthy controls. During the second exposure, there were significant increases only for eosinophils, IL-5, IL-6, and macrophage inflammatory protein-1 $\beta$ . Badorrek et al concluded that these biomarkers identified in nasal lavage and secretions could be used to monitor the immunologic efficacy of AR treatments during trials using CACFs.<sup>26</sup>

### Nasal Allergen Challenge

In addition to CACFs, the nasal allergen challenge (NAC) model is another well-established experimental model of AR.<sup>27</sup> NACs involve the direct application of allergen into the nose of study participants using a nasal spray device. By focusing on a smaller number of participants, extensive follow-up and more comprehensive biological sampling can be performed compared with studies using CACFs.<sup>27</sup> During the past 2 years, NAC studies published in the *Annals* have focused on evaluating alternative methods of direct allergen delivery and investigating the underlying inflammatory mechanisms after a direct allergen challenge.

A pilot study conducted by Ellis et al<sup>28</sup> sought to optimize the qualifying criteria, concentration of delivered allergen, and reliability and repeatability of symptoms using the NAC model.

Individuals with ragweed pollen-induced AR were enrolled into the study, consisting of a screening visit and a challenge visit. During screening, participants underwent a titrated allergen challenge, receiving increasing doses of allergen until 1 of 3 qualifying symptom criteria were met (using TNSS and/or PNIF). They received their qualifying dose (qualifying allergen concentration [QAC]) during the challenge visit. In a separate arm of the study, participants instead received a cumulative concentration of all screening doses received (cumulative allergen concentration [CAC]) during the challenge visit. For the 2 arms, participants recorded their TNSS and PNIF at identical time points during the challenge visit. Ellis et al demonstrated that the inclusion of TNSS and PNIF in the qualifying criteria led to the most repeatable peak symptoms achieved between screening and challenge visits. However, when comparing QAC and CAC protocols using similar qualifying criteria, a decrease in peak TNSS and PNIF was observed during the challenge compared with screening for the 2 measures.<sup>28</sup> This suggests that further modification to the concentration of allergen delivered might be warranted. These questions were addressed in a follow-up study by Soliman et al.<sup>29</sup> A third iteration of the protocol, in which participants were instead challenged with the CAC followed by the QAC alone 15 minutes later, was evaluated to determine whether this protocol improved symptom repeatability between visits (multiple CAC). Using similar protocols, Soliman et al<sup>29</sup> demonstrated improved repeatability in peak TNSS and PNIF achieved between screening and challenge visits when using the multiple CAC protocol compared with the CAC and QAC protocols. These 2 studies illustrate the complexity of the NAC model, highlighting the need for protocol optimization before using the model to evaluate antiallergic medication.

Recent studies have used the NAC model to characterize local and systemic inflammatory mechanisms in patients with AR. Using the aforementioned CAC protocol, Soliman et al<sup>30</sup> characterized local nasal cytokine responses in participants with AR after allergen challenge. Participants allergic and not allergic to ragweed were enrolled and challenged with ragweed pollen extract. Nasal secretions were collected at baseline and 1 and 6 hours after NAC to assess cytokine levels. Proinflammatory cytokines such as IL-5, IL-6, and IL-13 were significantly upregulated after the NAC in patients allergic to ragweed compared with controls.<sup>30</sup> Another study conducted by Tomljenovic et al<sup>31</sup> used the NAC model to assess the role of the nasal-ocular reflex in enhancing ocular symptoms in patients with seasonal AR after specific and nonspecific challenges. Patients with seasonal AR were enrolled and underwent 3 consecutive NACs to low-dose allergen (grass or ragweed), histamine, and 2% hypertonic saline (irritant). Challenges were separated by 24-hour (after allergen) and 48-hour (after histamine) washouts. Tears and nasal lavage samples were collected after each challenge. A significant increase in substance P was observed in tears after histamine and saline challenges, which positively correlated with ocular itching. Schirmer scores (objective measure of ocular lacrimation) also were higher after histamine and saline challenges compared with allergen challenge. Tomljenovic et al concluded that the release of neural mediators after nonspecific stimuli might enhance ocular symptoms in allergic patients already primed (after low-dose allergen challenge) and without allergen exposure.<sup>31</sup> A recent 2-way crossover study was conducted to assess the role of peripheral cell mitochondrial function in patients with grass pollen-induced AR after a NAC with grass pollen extract. Blood samples were collected at baseline and 6 and 24 hours after challenge, peripheral blood mononuclear cells were isolated, and mitochondrial function was measured. After allergen exposure, Qi et al<sup>32</sup> observed a significant impairment in peripheral blood mononuclear cell mitochondrial function at 6 hours, which returned to baseline at 24 hours after challenge. Conversely, peripheral blood eosinophils increased at 6 and 24 hours and were negatively correlated with peripheral blood

mononuclear cell mitochondrial function at 6 hours.<sup>32</sup> These results highlight the interesting and possible role of peripheral cell mitochondrial function in patients with AR.

## Conclusion

In summary, many notable publications regarding advances in rhinitis research were included in the 2015 to 2017 issues of the *Annals*. These publications focused on identifying early-life predictors of AR, advancing our understanding of the mechanisms underlying CRS and its impact on asthma management, and providing insight into the different applications of CACFs and NACs as robust experimental models for studying AR pathogenesis.

## References

- [1] Greiner AN, Hellings PW, Rotiroti G, et al. Allergic rhinitis. *Lancet*. 2011;378:2112–2122.
- [2] Brożek JL, Bousquet J, Agache I, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines—2016 revision. *J Allergy Clin Immunol*. 2017;140:950–958.
- [3] Codispoti CD, Bernstein DI, Levin L, et al. Early-life mold and tree sensitivity is associated with allergic eosinophilic rhinitis at 4 years of age. *Ann Allergy Asthma Immunol*. 2015;114:193–198, e4.
- [4] Lee E, Lee SH, Kwon J-W, et al. A rhinitis phenotype associated with increased development of bronchial hyperresponsiveness and asthma in children. *Ann Allergy Asthma Immunol*. 2016;117:21–28, e1.
- [5] Gelardi M, Iannuzzi L, Quaranta N, et al. Nasal cytology: practical aspects and clinical relevance. *Clin Exp Allergy*. 2016;46:785–792.
- [6] Akdis CA, Bachert C, Cingi C, et al. Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol*. 2013;131:1479–1490.
- [7] Zhang N, Zele TV, Perez-Novo C, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol*. 2008;122:961–968.
- [8] Kim J-W, Hong S-L, Kim Y-K, et al. Histological and immunological features of non-eosinophilic nasal polyps. *Otolaryngol Head Neck Surg*. 2007;137:925–930.
- [9] Sakuma Y, Ishitoya J, Komatsu M, et al. New clinical diagnostic criteria for eosinophilic chronic rhinosinusitis. *Auris Nasus Larynx*. 2011;38:583–588.
- [10] Iinuma T, Okamoto Y, Yamamoto H, et al. Interleukin-25 and mucosal T cells in noneosinophilic and eosinophilic chronic rhinosinusitis. *Ann Allergy Asthma Immunol*. 2015;114:289–298.
- [11] Kagoya R, Kondo K, Baba S, et al. Correlation of basophil infiltration in nasal polyps with the severity of chronic rhinosinusitis. *Ann Allergy Asthma Immunol*. 2015;114:30–35.
- [12] Baba S, Kondo K, Suzukawa M, et al. Distribution, subtype population, and IgE positivity of mast cells in chronic rhinosinusitis with nasal polyps. *Ann Allergy Asthma Immunol*. 2017;119:120–128.
- [13] Tosca MA, Riccio AM, Marseglia GL, et al. Nasal endoscopy in asthmatic children: assessment of rhinosinusitis and adenoiditis incidence, correlations with cytology and microbiology. *Clin Exp Allergy*. 2001;31:609–615.
- [14] Promsopa C, Kansara S, Citardi MJ, et al. Prevalence of confirmed asthma varies in chronic rhinosinusitis subtypes. *Int Forum Allergy Rhinol*. 2016;6:373–377.
- [15] Parsons DS, Phillips SE. Functional endoscopic surgery in children: a retrospective analysis of results. *Laryngoscope*. 1993;103:899–903.
- [16] Oliveira CAA, Solé D, Naspitz CK, et al. Improvement of bronchial hyperresponsiveness in asthmatic children treated for concomitant sinusitis. *Ann Allergy Asthma Immunol*. 1997;79:70–74.
- [17] Tosca MA, Cosentino C, Pallestrini E, et al. Improvement of clinical and immunopathologic parameters in asthmatic children treated for concomitant chronic rhinosinusitis. *Ann Allergy Asthma Immunol*. 2003;91:71–78.
- [18] Anfuso A, Ramadan H, Terrell A, et al. Sinus and adenoid inflammation in children with chronic rhinosinusitis and asthma. *Ann Allergy Asthma Immunol*. 2015;114:103–110.
- [19] Phillips KM, Hoehle LP, Caradonna DS, et al. Association of severity of chronic rhinosinusitis with degree of comorbid asthma control. *Ann Allergy Asthma Immunol*. 2016;117:651–654.
- [20] Ellis AK, North ML, Walker T, et al. Environmental exposure unit: a sensitive, specific, and reproducible methodology for allergen challenge. *Ann Allergy Asthma Immunol*. 2013;111:323–328.
- [21] North ML, Soliman M, Walker T, et al. Controlled allergen challenge facilities and their unique contributions to allergic rhinitis research. *Curr Allergy Asthma Rep*. 2015;15:11.
- [22] Pfaar O, Calderon MA, Andrews CP, et al. Allergen exposure chambers: harmonizing current concepts and projecting the needs for the future—an EAACI Position Paper. *Allergy*. 2017;72:1035–1042.
- [23] Soliman M, Ellis AK. Phenotyping allergic rhinitis as early- or dual-phase responses using the environmental exposure unit. *Ann Allergy Asthma Immunol*. 2015;114:344–345.
- [24] Jacobs RL, Ramirez DA, Rather CG, et al. Redness response phenotypes of allergic conjunctivitis in an allergen challenge chamber. *Ann Allergy Asthma Immunol*. 2017;118:86–93, e2.
- [25] Okuma Y, Okamoto Y, Yonekura S, et al. Persistent nasal symptoms and mediator release after continuous pollen exposure in an environmental challenge chamber. *Ann Allergy Asthma Immunol*. 2016;117:150–157.
- [26] Badorrek P, Müller M, Koch W, et al. Specificity and reproducibility of nasal biomarkers in patients with allergic rhinitis after allergen challenge chamber exposure. *Ann Allergy Asthma Immunol*. 2017;118:290–297.
- [27] Soliman M, North ML, Steacy LM, et al. Nasal allergen challenge studies of allergic rhinitis: a guide for the practicing clinician. *Ann Allergy Asthma Immunol*. 2014;113:250–256.
- [28] Ellis AK, Soliman M, Steacy L, et al. The Allergic Rhinitis–Clinical Investigator Collaborative (AR-CIC): nasal allergen challenge protocol optimization for studying AR pathophysiology and evaluating novel therapies. *Allergy Asthma Clin Immunol*. 2015;11:16.
- [29] Soliman M, Steacy L, Thiele J, et al. Administering multiple cumulative allergen challenges: an alternative nasal allergen challenge protocol for the Allergic Rhinitis–Clinical Investigator Collaborative. *Ann Allergy Asthma Immunol*. 2016;117:326–328.
- [30] Soliman M, Thiele J, Boulet L-P, et al. Mapping the local inflammatory cytokine secretion following a cumulative allergen dose using the Allergic Rhinitis Clinical Investigator Collaborative Nasal Allergen Challenge model. Poster presented at: European Academy of Allergy and Clinical Immunology Annual Congress; A1059; June 6–10, 2015; Barcelona, Spain.
- [31] Tomljenovic D, Baudoin T, Megla ZB, et al. Nasal and ocular responses after specific and nonspecific nasal challenges in seasonal allergic rhinitis. *Ann Allergy Asthma Immunol*. 2016;116:199–205.
- [32] Qi S, Barnig C, Charles A-L, et al. Effect of nasal allergen challenge in allergic rhinitis on mitochondrial function of peripheral blood mononuclear cells. *Ann Allergy Asthma Immunol*. 2017;118:367–369.