

Redness response phenotypes of allergic conjunctivitis in an allergen challenge chamber



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ARTICLE INFO

Article history:

Received for publication July 27, 2016.

Received in revised form October 24, 2016.

Accepted for publication October 26, 2016.

ABSTRACT

Background: There are few direct data concerning symptom dynamics of allergic conjunctivitis (AC) in an allergen challenge chamber (ACC).

Objective: To determine the AC dynamics on subsequent exposures to ragweed pollen (RW) in individuals with allergic rhinitis in an ACC. To determine whether consecutive exposures in an ACC have any persistent detrimental ocular physical effects.

Methods: Participants underwent 3 exposures to RW in an ACC. Ocular symptoms of itching and tearing were self-assessed. Ocular redness and lid swelling were assessed by trained ophthalmic technicians. Complete ophthalmic examinations (COEs) were performed by an ophthalmologist.

Results: A total of 188 of 201 participants (93%) developed an ocular redness score of 2 or more in each eye in ACC exposure 1. Reproducibility of redness occurred in approximately 70% of individuals completing ACC exposures 1 through 3. There were no significant changes between baseline COE and end of study COE. Phenotypes were identified by redness responses during and after exposure. Baseline total ocular symptom scores, at 24 hours after a priming exposure, were identified as late-phase reactions rather than enhanced sensitivity.

Conclusion: When assessed by trained professionals, AC was present with a very high frequency in selected individuals allergic to RW monitored in an ACC. Intrasubject reproducibility of redness was consistent across 3 ACC allergen exposures. Phenotypes were identified as early-phase responses, protracted early-phase responses, dual responses, and late-phase responses.

Trial Registration: clinicaltrials.gov Identifier: NCT02079649.

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Introduction

Allergic rhinoconjunctivitis (ARC) is a common disorder, affecting up to 40% of the general population in the some westernized countries.¹ Signs and symptoms of the ocular component of ARC, including early-phase responses (EPRs) of itching, tearing, redness, lid swelling, and chemosis, that transition into late-phase responses (LPRs) may cause significant morbidity. However, most natural seasonal studies of ARC have focused on the rhinitis component with less emphasis on ocular itching, tearing, and redness and with almost no consideration of lid swelling, chemosis, and hypererythema, which can be the most disturbing of ocular

symptoms. There are few data concerning the frequency and severity of ocular signs and symptoms occurring within the controlled environment of an allergen challenge chamber (ACC) in which relatively high levels of an allergen are sustained for several hours. Likewise, there are neither data concerning LPRs nor any data regarding adverse physical effects extending for a period beyond these sustained exposures. Ellis et al² used a quality of life (QOL) survey to evaluate the aftereffects of a 14-hour allergen exposure. Although QOL decreased after the prolonged exposure, it had returned to or improved over baseline within 2.5 weeks. No data were gathered concerning physical effects after exposure.

As very specific forms of allergy intervention evolve, use of an ACC will be necessary to define the various clinical characteristics of these approaches. To our knowledge, there are no published data regarding the dynamics of ocular signs or symptoms elicited in an ACC or the lasting physical effects of such exposures. Careful selection of participants for interventional studies is critical to avoid either enrollment of those with heterogeneous phenotypes of

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Disclosures: Drs Jacobs, Andrews, and Ramirez are co-owners of the Biogenics Research Chamber. Ms Rather is the executive director and lead coordinator of chamber operations at Biogenics Research Chamber.

Funding Sources: This study was funded by Alcon Research Ltd, Fort Worth, Texas.

<http://dx.doi.org/10.1016/j.anai.2016.10.023>

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responsiveness or unequal distribution of these phenotypes within treatment arms. We endeavored to further understand the dynamics of ocular symptoms elicited during chamber exposures by specifically identifying the response phenotypes and any lingering physical effects after intense, repeated, prolonged exposures. As suggested by the workshop at the National Institute of Health in 2010,³ we have evaluated the ocular manifestations, with particular focus on redness.

Methods

Selection of Study Participants

The study population included 224 individuals who consented at a single site. Screened participants, selected primarily from allergy databases, were 18 years or older with a history of ARC during the ragweed (RW) season and a skin prick test (SPT) wheal to short ragweed allergen 5 mm or larger compared with the saline control. Participants had a total nasal symptom score of 7 of 12 or greater on a Likert scale during prior natural seasonal studies. There were no natural season ocular severity criteria for participant selection. All participants were ACC allergen exposure naive for recording ocular symptoms. The complete inclusion and exclusion criteria are presented in [eTable 1](#). The protocol, amendments, and informed consent documents were approved by Sterling Institutional Review Board (Atlanta, GA). This clinical study was conducted in accordance with Good Clinical Practice guidelines and applicable regulations.

Ocular Symptom Scoring

Ocular signs and symptoms of redness and lid swelling were assessed by trained ophthalmic technicians ([eMethods](#)). Redness was measured using a validated bulbar redness scale⁴ ([Fig 1](#)). Chemosis was evaluated and scored by ophthalmologists, and itching and tearing were assessed by the study participants ([eMethods](#)).

Study Design

An overview of the study design is shown in [Figure 2](#). This study was performed during from July to August when no pollen from any source was detected in San Antonio, Texas.

Screening Visit

Screening consisted of consent, medical history, physical examination, SPT, spirometry, and, when appropriate, a urine pregnancy test.

ACC Screening Exposure (Exposure 1) (Day 1)

Qualifying participants entered the ACC for 3 hours of exposure to RW at a mean (SD) pollen count of 3,500 (700) grains/m³, a density used for all subsequent ACC exposures. Trained ophthalmic technicians performed staff-rated ocular assessments, including ocular redness and lid swelling, and participants self-assessed ocular itching and tearing at 30-minute intervals. To continue in the study, participants were required to score 2 or greater on staff-assessed ocular redness in at least 1 region (nasal or temporal) in each eye within the 3-hour period of exposure.

Complete Ophthalmic Examination Visit (Days 4–7)

After chamber exposure 1, a complete ophthalmic examination (COE), including Early Treatment Diabetic Retinopathy Study

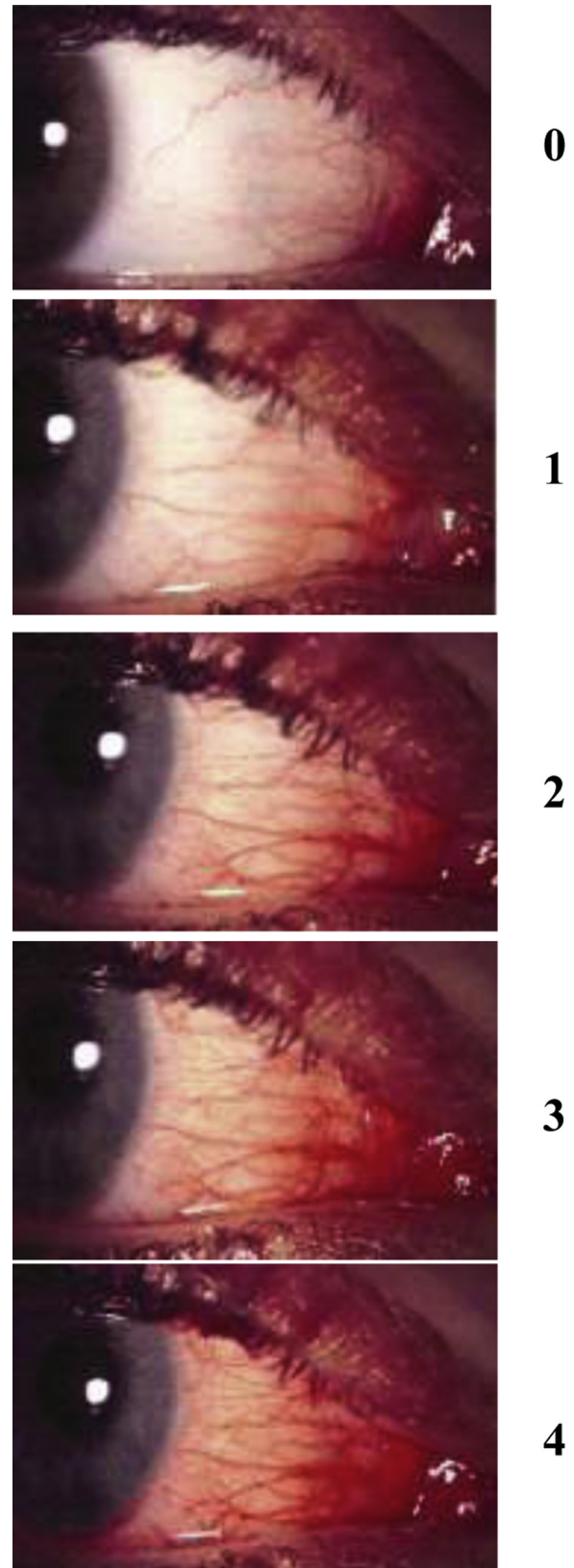


Figure 1. Ocular signs and symptoms assessments. Separate ratings for temporal and nasal regions 1 through 4 in each eye. Modified from the validated bulbar redness 5 scale.

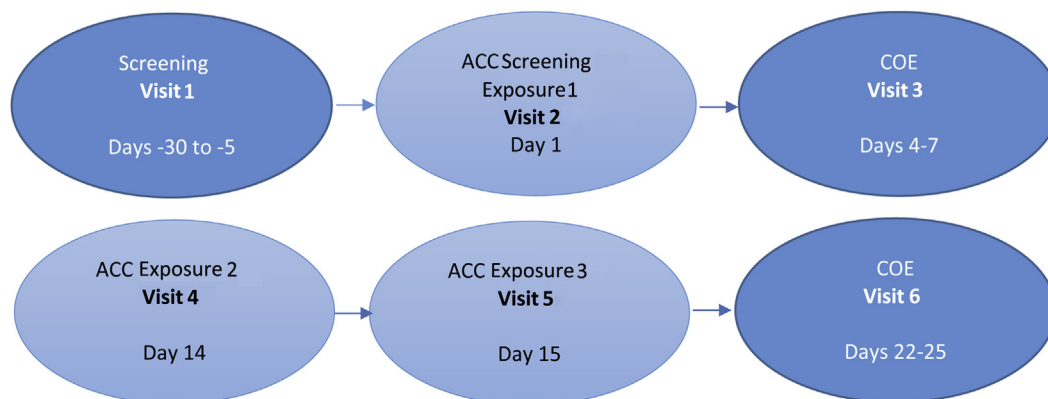


Figure 2. Schematic of the study. ACC indicates allergen challenge chamber; COE, complete ophthalmic examination.

(ETDRS) visual acuity, slitlamp examination, intraocular pressure (IOP) measurement, corneal edema or haze, and a dilated fundus examination, was performed by an ophthalmologist. Participants without any significant ocular findings were qualified for continuation.

ACC Exposure 2 (Day 14)

Before participants entering the ACC, trained ophthalmic technicians performed ocular assessments of ocular redness and lid swelling. Participants recorded ocular assessments of itching and tearing. If ocular redness (in any region) was greater than 1 during the pre-ACC assessments, participants were rescheduled at the discretion of the investigator.

Participants then entered the ACC for a 3-hour pollen exposure. Technician assessments and participant self-assessments were performed at the same time points as in exposure 1. To qualify for continuation, participants were required to score 2 or higher for staff-assessed ocular redness in at least 1 region (nasal or temporal) in each eye within the 3-hour exposure.

ACC Exposure 3 (Day 15)

Before participants entered the ACC, trained staff performed ocular assessments for LPRs, including ocular redness and lid swelling, and participants recorded ocular symptoms. During exposure 3, technician assessments and participant self-assessments were performed at the same time points as in exposures 1 and 2. After the exposure, participants were moved outside the pollen exposure chamber into a clean chamber. An ophthalmologist performed a chemosis assessment using a slitlamp. Participants were monitored in the clean chamber for an additional duration of 7 hours. During this time, staff-rated ocular assessments and participant self-rated assessments were conducted at the following time points: at 3.5 hours (half hour after entering the clean room) and every hour from hours 4 through 10.

Postexposure COE Visit (Days 22–25)

Participants returned 7 to 10 days after exposure 3 for a COE identical to the initial examination.

Statistical Analysis

A *t* test was used to study symptoms score changes from exposure 1 to 2 and 2 to 3. A paired *t* test was used to assess whether the change from exposure 1 to 2 differs from that from exposure 2 to 3. The above analysis was used for both mean and maximal scores. Within each exposure, a 2-way analysis of variance model was built, with one factor being time and the other factor

being phenotype. An interaction term was included, and repeated measures were accounted for in each participant. Contrasts were estimated, using Tukey correction, separately within each of the time points of interest.

Results

Study Population

A total of 224 RW-sensitive individuals consented at screening. Fifty-nine individuals failed to meet inclusion criteria or withdrew consent. Thirteen of 201 individuals at exposure 1 (6.5%) failed to develop the redness criteria needed to continue, and 1 individual developed severe ocular symptoms, requiring withdrawal by the principal investigator. Of the 224 participants who consented to participate in the study, 165 completed the study. Ninety of 165 (54.5%) were female, 146 were white (88.5%), 104 were Hispanic (63%), and 17 (10.3%) were African American. The mean age was 44.0 years (range, 19–77 years).

Skin Prick Tests

A 5-mm or larger wheal on a SPT was designated an inclusion criteria. SPT wheals measuring 5 to 9 mm, 10 to 14 mm, and greater than 15 mm was observed in 137, 20, and 8 participants, respectively. There was no correlation between skin test size and severity of ocular symptoms.

Chemosis

Chemosis was scored in each eye by slitlamp examination at 21 hours after exposure 2 and after allergen exposure 3. The mean The mean after chemosis did correlate with the mean redness (correlation, -0.174 ; $P = .03$). The maximum after chemosis did not correlate with maximum redness (correlation, -0.123 ; $P = .12$). The mean and maximum after chemosis did not correlate with the mean and maximum swelling (mean swelling correlation, -0.039 ; $P = .62$; maximum swelling correlation, -0.018 ; $P = .8$).

Consistency of Total Ocular Symptom Scores

Participants were compared by their responses during exposures 1, 2, and 3 to determine reproducibility of redness, swelling, itching, and tearing. Participants scoring high/high/high or low/low/low for the various symptoms were considered reproducible (Table 1). There were some statistical differences at time points between exposures I to III in both total ocular symptom score (TOSS) (Fig 3) and redness (Fig 4).

Table 1
Ocular Sign and Symptom Consistency

Exposure 1 2 3	Redness, %	Lid swell, %	Itching, %	Tearing or watering, %
H H H	4.2	0.0	46.7	12.7
H H L	0.6	0.0	10.9	3.0
H L H	2.4	0.0	4.8	1.8
H L L	7.3	0.6	10.3	7.3
L H H	4.2	0.0	5.5	3.6
L H L	0.6	0.0	3.0	3.6
L L H	12.1	0.6	1.8	3.0
L L L	68.5	98.8	17.0	64.8

Abbreviations: H, high (mean symptom score >2); L, low (mean symptom score of 0–2).

Phenotypes of Redness Responses

Using phenotyping of allergic rhinitis (AR) within an environmental exposure unit as a model,⁵ we sought to establish similar phenotyping of redness of allergic rhinitis (AC) under somewhat different conditions and criteria. We defined redness responses during exposure 3 thusly: EPRs were defined as a 50% or greater decrease in redness by hour 7 that did not increase and returned to baseline by hour 10 of exposure 3; protracted early-phase responses (pEPRs) were defined as a 50% or greater reduction in redness by hour 7 with most not returning to baseline by hour 10; dual responses (DRs) were defined as a decrease of 50% or more by hours 7 to 9 followed by a 50% or greater increase in redness by hour 10; and LPRs were defined as a 50% or greater increase over baseline redness of exposure 2 at 24 hours. All LPRs were identified as EPRs, pEPRs, or DRs during the pollen exposure and post-exposure observation period. By this classification, 66 of 165 participants (40%) had EPRs, with 29 scoring 0 for redness at 10 hours. Another 16 participants (9.7%) with EPRs experienced late-phase responses (EPR-LPRs). Fifty-two of 165 participants (32%) had pEPRs, with another group of 26 patients (16%) with pEPRs experiencing late-phase responses (pEPR-LPRs). Only 5 participants (3%) were classified as having DRs, with one of these also experiencing a late-phase response (DR-LPR). Overall, 43 participants (26%) were classified as having LPRs. There was a statistical difference ($P < .05$) between redness phenotypes in EPR and pEPR-LPRs at 180 minutes in exposure 1. In exposure 2, a baseline statistical difference between pEPR and pEPR-LPR ($P = .02$) and at 180 minutes a statistical difference between EPR and pEPR ($P < .001$) and EPR and pEPR-LPR ($P < .001$) was observed. In exposure 3, baseline significant differences were found between EPR and EPR-LPR ($P < .001$), between EPR and pEPR-LPR ($P < .0001$), between EPR-LPR and pEPR ($P < .001$), and between pEPR and pEPR-LPR ($P < .001$). At 180 minutes, significant differences were found between EPR and pEPR ($P < .001$) and between EPR and pEPR-LPR ($P < .001$). At 420 minutes, significant differences were found between EPR and pEPR, EPR and pEPR-LPR, EPR-LPR and pEPR, and EPR-LPR and pEPR-LPR ($P < .001$ for all). At 600 minutes, significant differences were found between all phenotypes ($P < .001$) except pEPR and pEPR-LPR ($P = .02$) and EPR and EPR-LPR ($P = .10$) (Fig 5).

After applying itching and tearing to the redness phenotypes, a somewhat different pattern evolves. There were no statistical differences between phenotypes in exposure 1 either at 15 or 180 minutes. At 180 minutes of exposure 2, significant differences were found between EPR and EPR-LPR ($P = .046$) and between EPR and pEPR ($P = .009$). In exposure 3, significant differences were found at baseline between EPR and EPR-LPR ($P < .001$), EPR and pEPR-LPR ($P = .02$), EPR-LPR and pEPR ($P < .001$), and pEPR and pEPR-LPR ($P = .048$). At 180 minutes of exposure 3, no significant differences were found. At 420 minutes, significant differences were found only between EPR and pEPR ($P < .001$). At 600 minutes, significant differences were again found between EPR and pEPR ($P = .004$) (Fig 6).

Complete Ophthalmic Examinations

No significant changes were detected in ETDRS visual acuity (eTable 2) or IOP (eTable 3) between screening and end-of-study COE. No participant experienced an increase from baseline in corneal edema or haze at the end-of-study COE. Dilated fundus examinations remained unchanged between COEs.

Adverse Events

No serious adverse events occurred. All reported adverse events were nonserious. One participant was removed from the study because of massive ocular swelling, possibly caused by direct ocular inoculation of pollen by rubbing the eyes.

Discussion

The percentage of allergic individuals, along with severity and duration of symptoms, is likely to increase because of evolving dynamics of the population and the environment. The potential causes include increase in hygienic environments, indoor lifestyles and global warming.^{6–12} Mindful of these dynamics, we sought to determine whether AC can be successfully studied in an ACC and to provide a framework for approaching the nuances of studying novel interventions.

In many cases, natural and environmental chamber clinical trials of AR have not considered AC or have evaluated ocular symptoms almost as an afterthought. Therefore, data concerning the frequency of occurrence, severity, complications, and morbidity are inconsistent. Data from the National Health and Nutrition Examination III survey,¹ the first epidemiologic study of ocular allergy in the United States, reported that up 40% of the population had experienced ocular symptoms at least once in their lifetime. Other data concerning the frequency of AC associated with AR, which are most often quoted, were obtained by 2 surveys conducted by telephone interviews¹³ and internet questionnaires.¹⁴ These surveys suggest that 40% of individuals with AR will also experience AC during the height of symptoms in a natural exposure in their worst month of the year. Other reports have found somewhat higher levels, ranging up to approximately 70% to 80%.^{15,16}

TOSS Consistency

Although many ACC studies of AR have been performed during the past 20 years, with use of repeated priming exposures, no reports have detailed the intrasubject response. The reported priming failure rates have ranged from 26% to 48% after 2 to 6 priming exposures^{17–19} to RW, implying that low responders tend to remain low. Many individuals, however, respond highly on the first priming exposure and respond equally well during the intervention exposure. Others will have increased symptoms with repeated priming exposures, revealing that symptoms can change, usually into the minimal qualifying range, and they become suspect individuals during the intervention.

In this study, no participant had evidence of prepriming from natural exposure at exposure 2. With LPRs, which were affected by exposure 3, staff-assessed ocular redness and lid swelling were consistent at the same level across 3 pollen exposures in more than 70% and 98% of participants, respectively (Table 1). Staff-assessment of these signs was used as a more reliable metric than participant-assessed redness and lid swelling because (1) redness is more pronounced in the inner and outer quadrants of the eye, making it difficult for participants to score themselves by mirror observation, and (2) technician assessments provide consistency among the participants. Participant-assessed tearing or watery eyes was also consistent across the 3 exposures at a high level, with itching showing more variation.

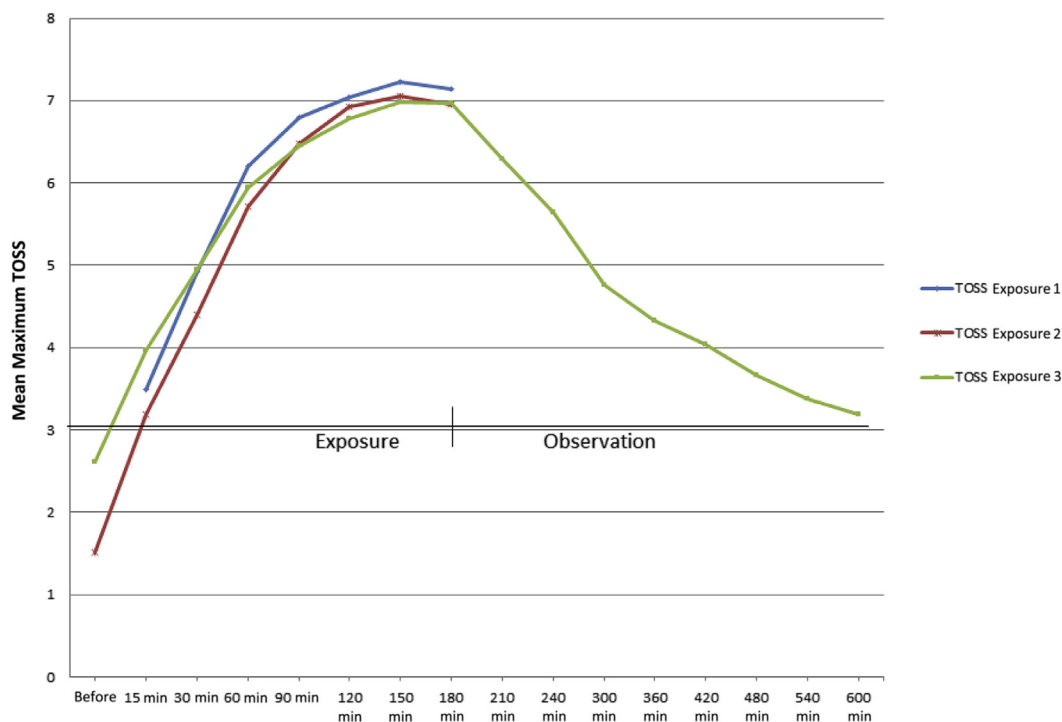


Figure 3. Maximum total ocular symptom score (TOSS) between exposures 1 and 3. Significant differences were found in the maximum TOSSs between exposures at isolated time points during the upward trajectory, but no statistical differences were found between the 3 exposures at plateau from 120 to 180 minutes.

EPR, pEPR, DR, and LPR

As seen in a population of patients with AR,⁵ similar phenotypes can be identified in the conjunctival symptoms.^{20–23} EPRs and LPRs have been previously identified in guinea pigs²⁰ USING direct ocular challenges, with EPRs occurring in a few minutes and LPRs

occurring within 3-, 9-, and 24-hour periods. All animals developed EPRs and LPRs with patterns described as biphasic (33%), multiphasic (25%), and prolonged (41%). Similar approaches²¹ have been performed in rye grass sensitive humans evaluated before challenge, after 20 minutes, hourly through 12 hours, and 24 hours after

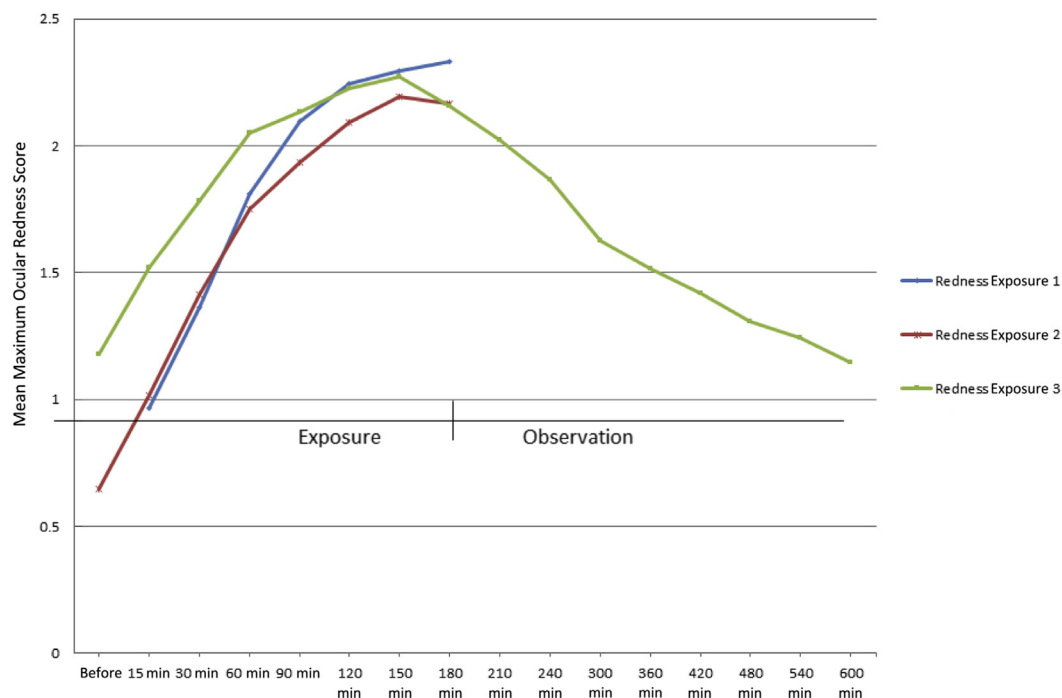


Figure 4. Mean maximum ocular redness between exposures 1 and 3. Significant differences were found between exposures 1 and 2 from the 90- to 180-minute periods ($P < .001$) and between exposures 2 and 3 only from baseline to the 120-minute period ($P < .001$).

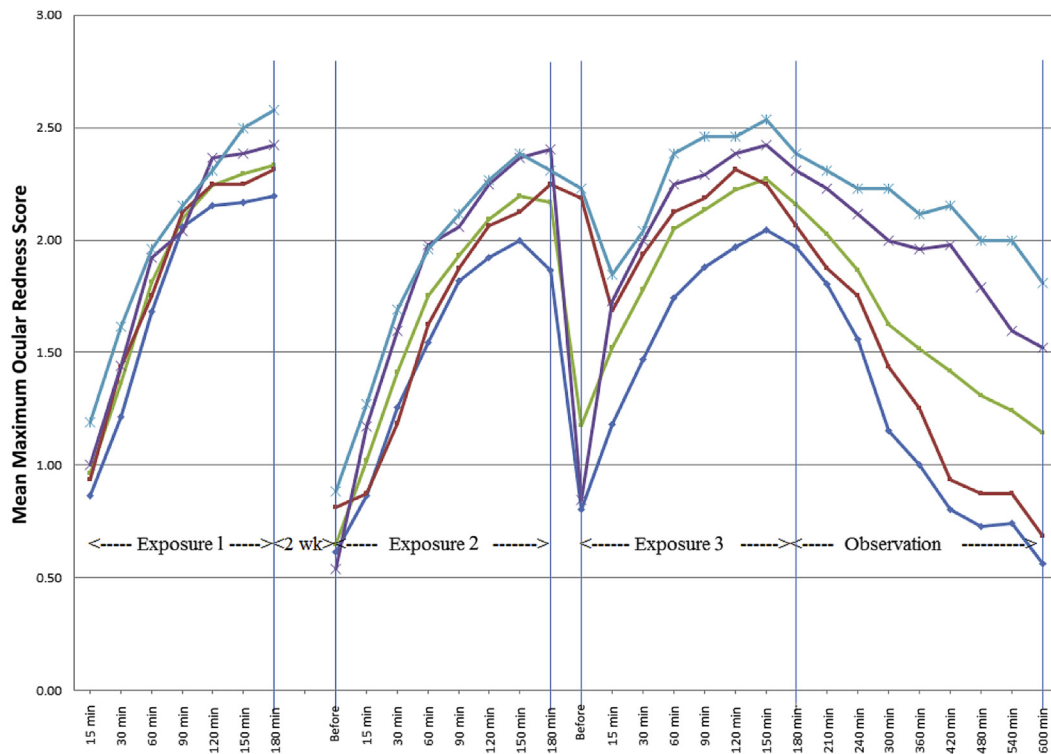


Figure 5. Exposures 1 through 3 by redness phenotypes.

the challenge. Results indicate a significant clinical reaction during the entire period with differing peaks in individuals. The inflammatory response in tear fluid was neutrophilic in the early phase and eosinophilic or lymphocytic in the late phase. Bacon et al.,²² again using a direct conjunctival challenge with either extracted mixed grass or *Dermatophagoides pteronyssinus*, reported an EPR (maximal at 20 minutes) associated with an increase in tear histamine and tryptase levels, which had returned to control levels at 40 minutes. An LPR was recognized at 6 hours with increased symptoms, a second peak in tear histamine and eosinophil cationic protein, but no tryptase. On biopsy, an infiltrate of several inflammatory cells, including mast cells, neutrophils, eosinophils, macrophages, and basophils, was reported. None of these individuals reported symptoms 24 hours after challenge.

In ACC exposures, the dynamics may be somewhat different than those identified with direct conjunctival challenges. ACC challenges are sustained for several hours at a fixed rate of exposure, which more closely mimics a natural exposure during high pollinating days. A report from the environmental exposure unit,⁵ using RW, defined phenotypes in AR using similar descriptions as those defined during direct conjunctival challenges. In that report, however, emphasis was on EPR, pEPR, and DR without defining LPR. In our study of AC, all participants responded within the 90- to 120-minute period with the entire spectrum of symptoms. There was consistency, based on phenotypes, across the 3 exposures regarding the level of redness (Fig 5), with descending intensity of pEPR-LPR, pEPR, EPR-LPR, and EPR.

A similar consistency was noted regarding TOSS (Fig 6) across the 3 chamber exposures, with descending intensity of EPR-LPR, pEPR, pEPR-LPR, and EPR. The pattern, however, of symptomatic expression during the observation period changed when considering TOSS because of the rapid decrease of the symptoms of itching and tearing, leading to a less clear separation of phenotypes. Itching, tearing, and redness are equally dominant symptoms of EPR. However, redness is the dominant symptom of LPR and,

therefore, the primary predictor. In comparison to the report by Solimen et al.,⁵ there was a similar rapid decrease in total nasal symptom score in those with EPRs as seen in TOSS in our study participants. However, use of total nasal symptom score rather than congestion (the dominant LPR of nasal symptoms) without an evaluation at the 24-hour mark reduced the ability to recognize LPRs in those individuals who may have had asthma exacerbation after the 12-hour time point. Only those individuals identified as having DRs were identified as having LPRs, with several in the EPR and pEPR groups unrecognized. The significance of recognizing response phenotypes may be important during study participant selection to maintain a balance in treatment arms. Mediators and cell types are somewhat different in these phenotypes. Interventions designed to counter specific biologic targets, or inflammatory pathways, may be quite effective in controlling a certain phenotype but much less so in controlling others. As an example, a trial of an intervention designed to counter the LPR would likely fail if individuals with EPRs (approximately 40%) are included because they do not react in the late phase. It is unclear whether a pEPR is in the realm of a LPR, simply an EPR that becomes prolonged, or a hybrid response. DRs as defined by this study were relatively rare and represent the classic definition of an EPR followed by a LPR within a certain time frame. However, both the EPR-LPRs and pEPR-LPRs are also DRs that are outside the defined time frames.

Priming

Priming (augmenting sensitivity) is a concept initiated by Connell²³ in the 1960s as he studied nasal reactivity by using pollen grains flecked directly into the nares. This concept of priming was adopted, by those using allergen chambers,² as nasal studies were initiated in controlled environments. Priming included up to 6 exposures,² usually 120 minutes in duration, when using a pollen out of season. However, there were diminishing returns recognized

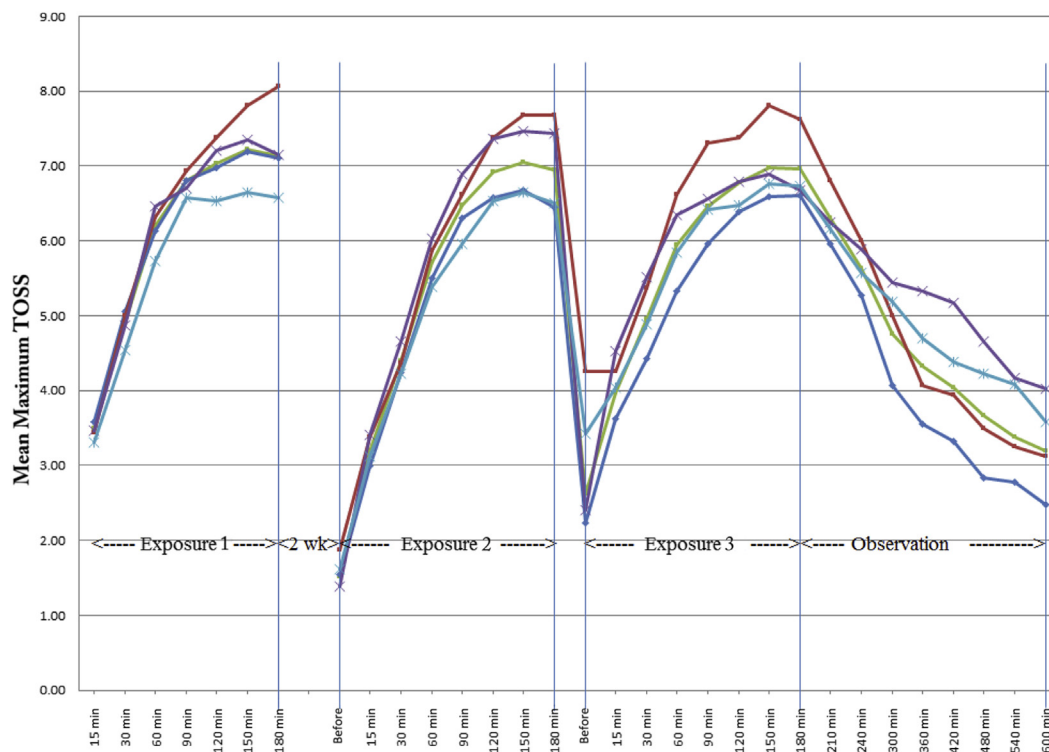


Figure 6. Total ocular symptom score (TOSS) sorted by redness phenotype during exposure 1 to 3.

during a RW study²⁴ in which 4 priming exposures were performed on sequential days and in which after the second exposure very few additional participants qualified.

In this study of AC, chamber exposure 1 was designed simply to identify nonresponders or low responders. No priming effects were noted during chamber exposure 2, 14 days after chamber exposure 1. In fact, symptom scores were numerically, but not statistically, slightly lower during exposure 2 compared with exposure 1. During exposure 3, only 1 day after exposure 2, baseline symptom scores were higher than baseline at exposure 2, and the trajectory of symptom scores was more robust than in exposure 2. However, the maximum TOSS score at the end of the exposure 3 period was similar to that of exposure 2. This finding suggests that the priming effect may simply be a manifestation of a LPR captured within a 24-hour period. We believe that a screening exposure, 7 or more days before an out-of-season pollen challenge, is adequate to eliminate nonresponders or low responders from the treatment cohort, particularly for studies in which procedures for biomarkers and immunologic assays are to be performed. However, if priming is believed to be necessary, it should be performed approximately 3 days before the primary challenge to avoid LPRs. Perennial allergen challenges should also require a screening exposure, with similar time constraints, but no priming should be required, assuming there is ongoing prepriming from exposures in the natural setting. Excessive priming, particularly, with perennial allergens may lead to initiation of low-level tolerance.²⁵

Physical Effects of Prolonged Consecutive Exposures

Despite some intense itching and redness leading to eye rubbing during the chamber exposures, there were no discernible ocular changes, other than the acute allergic condition studied, during the prolonged observation period and no change after COE regarding visual acuity, IOP, corneal edema, or haze. Dilated fundus examination remained unchanged between COEs, suggesting no prolonged ocular sequelae other than late-phase signs and symptoms.

In conclusion, redness with direct assessment by ophthalmic technicians and increases of TOSS occurred at a very high rate (93%) within the chamber setting. No priming effects were noted after a 2-week hiatus between exposures. Priming effects after 24 hours may simply be a LPR. Reproducibility of ocular symptoms was approximately 70% in individuals across 3 exposures. Phenotypes of EPRs, pEPRs, and DRs for redness were identified, with late phases occurring in all 3 groups. No prolonged ocular complications were identified as a result of consecutive, prolonged pollen exposures.

Ocular challenge within a controlled environment appears to have several advantages over other models: (1) ACC exposure more closely mimics natural exposures; (2) multiple people can be challenged as a cohort under identical circumstances; (3) transitions from EPR to pEPR to LPR can be better investigated simultaneously under controlled conditions; (4) with ongoing exposure, biologic specimens can be collected at any time point during the trajectory (upward, at the plateau, in the downward slope) or in the late phase; and (5) responses to interventions can be defined in the various phenotypes.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2016.10.023>.

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eMethods. Sign and Symptom Scoring

Ocular signs and symptoms of redness and lid swelling were assessed by trained ophthalmic technicians. Redness was measured using a validated bulbar redness scale¹ (Fig 1), with separate ratings recorded from 0 to 4 for the temporal and nasal regions in each eye. Lid swelling was assessed as levels 0 to 4 (level 0, none; level 1, edema in 1 quadrant of lids; level 2, definite alteration in lid folds; level 3, edema to lash margin; and level 4, lid closure). Chemosis was evaluated and scored by ophthalmologists. Chemosis was assessed as levels of 0 to 3 (level 0, none; level 1, visible with a slitlamp or penlight and involves primarily the inferior cul-de-sac; level 2, involves the bulbar conjunctiva and may need slitlamp

observation to confirm the presence of bulbar chemosis; and level 3, obvious with edematous conjunctiva spilling over the lower eyelid margin is visible using a penlight and not requiring a slitlamp). Itching and tearing were assessed by the study participants. Itching was assessed on a Likert scale of 0 to 4 (0, none; 1, tickling sensation involving one or more corners of the eye; 2, all over tickling sensation; 3, moderate continuous itching with desire to rub; and 4, severe itching with irresistible urge to rub). Tearing was assessed on a Likert scale of 0 to 3 (0, none; 1, mild, eye feels slightly watery; 2, moderate, blows nose occasionally; and 3, severe, tears roll down cheeks). Total ocular symptoms score is defined as redness, itching, and tearing.

eTable 1
Inclusion and Exclusion Criteria

Inclusion Criteria
1. Provide written informed consent.
2. Age of 18 years or older.
3. Best-corrected visual acuity of 55 or greater in each eye as measured by Early Treatment Diabetic Retinopathy Study (letters read method).
4. Ability and willingness to comply with study protocol and follow protocol instructions.
5. Ability to avoid any of the topical ocular or systemic medications listed in the Prohibited Medication Table during the entire study period. Appropriate washout periods before the screen visit are provided in the Prohibited Medication Table. Acetaminophen less than 2 g/d as needed is allowed.
6. Positive skin prick test results for short ragweed allergen (≥5-mm wheal greater than the diluent after skin prick testing) within 12 months before the screen visit.
7. Score of 2 or higher for staff-assessed ocular redness in at least one region (nasal or temporal) in each eye within the 3-hour period of allergen exposure 1 in the allergen challenge chamber.
Exclusion Criteria
1. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception.
2. Presence of any ocular infection (bacterial, viral or fungal) or history of ocular herpes (simplex or zoster) or adenoviral infection in either eye or have had any ocular infection diagnosed by a physician or the participant within past 30 days of screen visit.
3. Presence of glaucoma, intraocular pressure greater than 21 mm Hg at examination, and history of or diagnosis of glaucoma or ocular hypertension. Presence of blepharitis, active rosacea affecting the ocular adnexa, meibomian gland dysfunction, follicular conjunctivitis, intraocular inflammation, preauricular lymphadenopathy, or any other ophthalmic abnormality that may affect the study outcomes.
4. Corneal conditions affecting the corneal structure.
5. Contact lens wear within 7 days before the screen visit or unwilling to discontinue contact lens wear during the study period.
6. Any ocular surgery, including ocular laser procedures, within 1 year of the screen visit.
7. Current or recent (<6 months before the screen visit) history of severe, unstable, or uncontrolled autoimmune, neurologic, cardiovascular, hematologic, hepatic, renal, psychological, respiratory, gastrointestinal, and/or immunologic disease or evidence of other diseases based on a review of medical history and/or physical examination (including vital signs, electrocardiogram, clinical laboratory test results, and body mass index) that, in the opinion of the investigator, would preclude safe subject participation in the study.
8. Participation in any investigational study within 30 days of the screen visit or concomitantly with this study.
9. Confirmed (by physician or optometrist) diagnosis of dry eye.
10. Physician-diagnosed asthma (except inactive childhood asthma and exercise-induced asthma).
11. Ratio of forced expiratory volume in 1 second to forced vital capacity less than 80% predicted as determined by spirometry at screening visit.

eTable 2Visual Acuity Change from Baseline^a

Maximum change	No. (%)
Letter increase >14	1/162 (0.6)
Letter increase of 10–14	0
Letter increase of 5–9	9/162 (5.6)
±4 Letters	121/162 (74.7)
Letter decrease of 5–9	28/162 (17.3)
Letter decrease of 10–14	2/162 (1.2)
Letter decrease >14 letters	0

^aEarly Treatment Diabetic Retinopathy Study visual screening was performed by ophthalmic technicians.

eTable 3Maximum Change of Intraocular Pressure from Baseline^a

Maximum change	No. (%)
Increase >30 mm Hg	0
Increase of 21–30 mm Hg	0
Increase of 11–20 mm Hg	0
Increase of 6–10 mm Hg	4/161 (2.5)
±5 mm Hg	148/161 (92.0)
Decrease of 6–10 mm Hg	9/161 (5.5)
Decrease of 11–20 mm Hg	0
Decrease of 21–30 mm Hg	0

^aAll intraocular pressures were measured with a calibrated Haag-Streit 870 Tonometer.