Cockroach sensitization mitigates allergic rhinoconjunctivitis symptom severity in patients allergic to house dust mites and pollen

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Background: Modifiers of symptom severity in patients with allergic rhinoconjunctivitis (AR) are imprecisely characterized. The hygiene hypothesis implicates childhood microbial exposure as a protective factor. Cockroach sensitization (C+) might be a proxy for microbial exposure.

Objective: We sought to determine whether C+ assayed by means of skin prick tests influenced AR symptom severity in controlled and natural settings.

Methods: Total symptom scores (TSSs) were recorded by 21 participants with house dust mite allergy (M+) in the natural setting and during repeated exposures of 3 hours per day to house dust mite allergen in an allergen challenge chamber (ACC). In M+ participants the peripheral blood and nasal cells were assayed for T-cell activation and transcriptomic profiles (by using RNA sequencing), respectively. Participants allergic to mountain cedar (n = 21), oak (n = 34), and ragweed (n = 23) recorded TSSs during separate out-of-season exposures to these pollens (any pollen sensitization [P+]) in the ACC; a subset recorded TSSs in the pollination seasons.

Results: The hierarchy of TSSs (highest to lowest) among M+ participants tracked the following skin prick test sensitization statuses: M+P+C− > M+P+C+ > M+P−C− > M+P−C+. In nasal cells and peripheral blood the immune/inflammatory responses were rapidly resolved in M+P+C+ compared with M+P+C− participants. Among those allergic to pollen, C+ was associated with a lower TSS during pollen challenges and the pollination season. After aggregated analysis of all 4 ACC studies, C+ status was associated with a 2.8-fold greater likelihood of a lower TSS compared with C− status (odds ratio, 2.78; 95% CI, 1.18-6.67; $P = .02$).

Conclusions: C+ status is associated with mitigation of AR symptom severity in adults with AR. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Cockroach sensitization, allergen challenge chamber, allergic rhinoconjunctivitis, T-cell activation, aeroallergens, house dust mite, skin prick test, pollen*

Allergic rhinoconjunctivitis (AR) is the most frequent IgE-mediated disease, with its prevalence reaching 40% in some surveys.[1-3] Depending on sensitization status, symptoms can be precipitated by seasonal/outdoor aeroallergens (eg, pollen), perennial/indoor aeroallergens (eg, house dust mites [HDMs] and cockroaches), or both.[4,5] Sensitization to the most common classes of aeroallergens is typically assessed by using skin prick test (SPT) responses.[4,5] However, even among patients with sensitization to multiple aeroallergens, the severity of AR symptoms varies widely.[4,5] Interindividual differences in AR symptom severity might relate to differences in aeroallergen concentrations in the environment. Another possibility is that even after controlling for concentration, sensitization to specific aeroallergens might modify the severity of AR symptoms elicited on exposure to another aeroallergen but not the sensitizing allergen. In other words, it is not the number of positive SPT responses *per se* but rather the presence of versus the lack of sensitization to specific aeroallergens that underpins interindividual variation in AR symptom severity.

We sought to determine whether sensitization to specific aeroallergens modifies AR symptom severity. To address this in the most controlled manner possible, we took advantage of 4 separate aeroallergen exposure studies previously conducted in an allergen challenge chamber (ACC). In these studies allergic adults volunteered for challenges to indoor or outdoor aeroallergens (HDMs, mountain cedar, Virginia live oak [VLO], and ragweed pollens).[6-8] Confounding factors were mitigated by 2 conditions. First, specific concentrations of the aeroallergens were delivered in a highly controlled environment, which elicited symptoms highly consistent with those observed in natural settings.[6-8] Second, studies were conducted when the concentration of tree pollens, a major inducer of AR symptoms in south Texas, was low. This strategy increased the likelihood that the symptoms elicited in the ACC were specifically related to the challenge aeroallergen and reduced the likelihood of confounding from exposure to other aeroallergens.[6,7]

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Abbreviations used
ACC: Allergen challenge chamber
AR: Allergic rhinoconjunctivitis
C+: Cockroach sensitization
GEE: Generalized estimating equations
HDM: House dust mite
ILC: Innate lymphoid cell
M+: House dust mite allergy
P+: Any pollen sensitization
SPT: Skin prick test
ssIgE: Serum specific IgE
TLR: Toll-like receptor
TSS: Total symptom score
VLO: Virginia live oak

Two sets of allergic individuals were evaluated. Study set 1 comprised participants who were allergic to HDM (M+) and challenged with HDM *(Dermatophagoides pteronyssinus)* in an ACC.[8] Three end points were evaluated: (1) AR symptom severity; (2) levels of peripheral blood T-cell activation, including CD4/CD8 T-cell ratio, a known correlate of T-cell activation[9-15]; and (3) gene expression responses in nasal cells determined by means of RNA sequencing. We investigated whether these end points differed in study participants who had a positive versus negative SPT response to pollens, cockroach allergens, or both. Study set 2 comprised participants who underwent separate exposures to mountain cedar, VLO, or ragweed pollen (any pollen sensitization [P+]) in an ACC.[6] In these participants we determined whether sensitization to cockroach influenced AR symptom severity.

Increasing evidence supports the hygiene hypothesis as a pathogenic basis for allergy, meaning that early-life exposure to certain allergens and bacteria might be associated with significant reductions in AR symptom severity and atopy.[5,16-18] Recently, Palm et al[5] revisited the tenets of the hygiene hypothesis. Although allergies are generally viewed as a mistargeted immune response that evolved to provide immunity to infectious agents, these authors posited that allergic immunity also has an important role in host defense against multiple noninfectious environmental insults.[5] Therefore we postulated that cockroach sensitization (C+) might be a proxy for childhood exposure to infectious and other environmental agents. We determined whether C+ status was associated with (1) mitigation of AR symptom severity in participants of study sets 1 and 2 in both the ACC and natural settings and (2) dampened immune activation/responses in study set 1 participants.

METHODS

Study participants for challenge studies in the ACC

The terms sensitization, sensitive, sensitivity, and reactivity are used interchangeably and refer to a positive SPT response (≥5 mm) to an aeroallergen. Allergic status or allergenicity refers to participants with a positive SPT response and symptoms on exposure to the specific allergen. Four separate groups of allergic participants were selected from a volunteer pool for the ACC studies conducted at the dates/months shown in Fig 1.[6-8] We first excluded participants who were not sensitive to the aeroallergen with which they had volunteered to be challenged in the ACC. Among participants with a positive SPT response for the challenge aeroallergen, we excluded those without symptoms consistent with AR to the challenge aeroallergen in the natural setting (for inclusion into the HDM challenge study) and pollination seasons (for inclusion into the pollen challenge studies). We also excluded those who did not meet the additional inclusion/exclusion criteria noted in Table E1 in this article's Online Repository at www.jacionline.org for the HDM challenge study[8]; similar inclusion/exclusion criteria applied to the pollen challenge studies.[6,7]

To mitigate confounding in the HDM challenge study, we excluded those allergic to ragweed because the HDM study was conducted in the fall. Ragweed was present in the environment, but tree pollens were not. Thus sensitization status to aeroallergens other than the challenge aeroallergen did not influence participant selection. For example, when participants were challenged with HDM, each had both a positive SPT response to HDM and symptoms consistent with HDM allergy in their natural environment. Selection for the HDM challenge study was not predicated on allergenicity to cockroach or pollens. Similarly, selection for pollen challenge studies required allergenicity to the challenge pollen and was not influenced by SPT responses or allergenicity to other aeroallergens.

Study set 1 comprised 21 participants who were allergic to HDM and completed all the phases of the HDM challenge study (Fig 2, *A*).[8] Participants in study set 1 were designated as M+ to reflect their allergenicity to HDM (Fig 1). Participants in study set 1 who were also allergic to pollens were designated as M+P+. Study set 2 included 3 separate groups of participants who were allergic to mountain cedar (n = 21), VLO (n = 34), and ragweed (n = 23) pollens, respectively. They were collectively designated as P+ to reflect their allergenicity to pollens. Symptom data collected in the mountain cedar, VLO, and ragweed pollination seasons were also available from 16, 17, and 23 participants, respectively, as described previously.[6,7] All participants with a positive SPT response to cockroach were designated as C+. Symptom responsiveness of C+ participants to cockroach was unknown because this can only be confirmed by a challenge study with cockroach aeroallergen.

The IntegReview Institutional Review Board (Austin, Tex) approved the studies. Allergy medications were prohibited during the study period (see Table E2 in this article's Online Repository at www.jacionline.org). All participants provided written informed consent. Studies were conducted according to Good Clinical Practice standards.

HDM and pollen challenge studies in the ACC

The HDM challenge study (study set 1) had 4 consecutive phases (Fig 2, *A*): (1) a 4-day run-in phase in the natural setting; (2) exposure to HDM allergen *(Dermatophagoides pteronyssinus)* for 3 hours each on 4 consecutive days (exposures 1-4, ACC-I phase); (3) a 38-day observation phase in the natural setting; and (4) 4 consecutive exposures (exposures 5-8) to HDM using conditions identical to those in ACC-I (ACC-II phase). Aeroallergen exposures in ACC-I and ACC-II commenced at 6 PM. Delivery of HDM powder and collection and quantification of HDM antigen in the ACC were performed as described previously[8] and summarized in the Methods section in this article's Online Repository at www.jacionline.org. Mountain cedar, VLO, and ragweed challenge studies (study set 2) included separate out-of-season exposures in the ACC to these 3 pollens for 3 hours each on 2 (mountain cedar and VLO) or 4 (ragweed) consecutive days, as described previously (Fig 1).[6,7]

Clinical end points in HDM and pollen challenge studies

In study set 1 each participant recorded nasal and ocular symptom scores on a Likert scale of 0 to 4 (see Table E3 in this article's Online Repository at www.jacionline.org). Total symptom scores (TSSs) were recorded as follows: (1) twice daily in the run-in and observation phases as reflective symptom scores; (2) before challenge with HDM (ie, baseline instantaneous TSS); and (3) every 30 minutes during exposure to HDM as instantaneous symptom scores.[8] Nasal symptoms were congestion, itching, sneezing, and rhinorrhea; ocular symptoms were itching, tearing, and redness. TSSs ranged from 0 to 28. In study set 2 participants recorded reflective TSSs in the pollination seasons twice daily; the protocol for recording instantaneous TSSs in the ACC was similar to that of study set 1, except that the symptoms were graded on a Likert scale of 0 to 3, for a TSS range of 0 to 21 (see Table E4 in this article's Online