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ORIGINAL ARTICLE

Rhinitis, Sinusitis and Upper Airway Disease

Large-scale provocation studies identify maladaptive responses to ubiquitous aeroallergens as a correlate of severe allergic rhinoconjunctivitis and asthma

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Abstract

Background: Allergic asthma (AA) and allergic rhinoconjunctivitis (ARC) are common comorbid environmentally triggered diseases. We hypothesized that severe AA/ARC reflects a maladaptive or unrestrained response to ubiquitous aeroallergens.

Methods: We performed provocation studies wherein six separate cohorts of persons (total n = 217) with ARC, with or without AA, were challenged once or more with fixed concentrations of seasonal or perennial aeroallergens in an aeroallergen challenge chamber (ACC).

Abbreviations: A, adaptive; AA, allergic asthma; ACC, aeroallergen challenge chamber; ACQ-7, asthma control questionnaire-7; ANOVA, analysis of variance; ARC, allergic rhinoconjunctivitis; AUC, area under the curve; COVID-19, coronavirus disease 2019; FDR, false discovery rate; FEV1, forced expiratory volume within one second; FLG, filaggrin; GEE, generalized estimating equation; GLM, generalized linear model; HDM, house dust mite; i, instantaneous; LRT, likelihood ratio test; M, maladaptive; MC, mountain cedar; OSM, oncostatin M; PARC, perennial ARC; POSTN, periostin; r, reflective; R, resilient; sIgE, HDM-specific IgE; SSS, summated symptom scores; TASS, total asthma symptom scores; Th2, T helper-2; TNSS, total nasal symptom scores; TOSS, total ocular symptom scores; TSS, total symptom scores; VLO, Virginia live oak.

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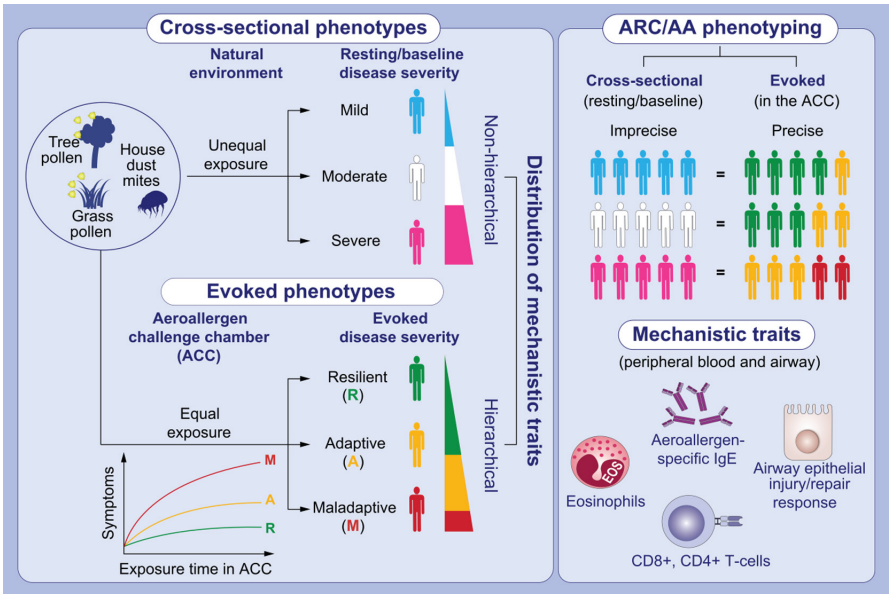
Results: Aeroallergen challenges elicited fully or partially restrained vs. unrestrained evoked symptom responsiveness, corresponding to the resilient and adaptive vs. maladaptive AA/ARC phenotypes, respectively. The maladaptive phenotype was evoked more commonly during challenge with a non-endemic versus endemic seasonal aeroallergen. In an AA cohort, symptom responses evoked after house dust mite (HDM) challenges vs. recorded in the natural environment were more accurate and precise predictors of asthma severity and control, lung function (FEV1), and mechanistic correlates of maladaptation. Correlates included elevated levels of peripheral blood CD4+ and CD8+ T-cells, eosinophils, and T-cell activation, as well as gene expression proxies for ineffectual epithelial injury/repair responses. Evoked symptom severity after HDM challenge appeared to be more closely related to levels of CD4+ and CD8+ T-cells than eosinophils, neutrophils, or HDM-specific IgE.

Conclusions: Provocation studies support the concept that resilience, adaptation, and maladaptation to environmental disease triggers calibrate AA/ARC severity. Despite the ubiquity of aeroallergens, in response to these disease triggers in controlled settings (ie, ACC), most atopic persons manifest the resilient or adaptive phenotype. Thus, ARC/AA disease progression may reflect the failure to preserve the resilient or adaptive phenotype. The triangulation of CD8+ T-cell activation, airway epithelial injury/repair processes and maladaptation in mediating AA disease severity needs more investigation.

KEYWORDS
aeroallergen challenge chamber, allergy, asthma, phenotypes, T-cells



GRAPHICAL ABSTRACT

In contrast to cross-sectional phenotyping in the natural environment, use of an ACC to control for level of exposure to an aeroallergen allows for identification of evoked phenotypes that track individuals with distinct symptom responsiveness. Cross-sectional phenotypes omit host responsiveness to aeroallergens and conflate persons with different evoked phenotypes. Mechanistic traits are more precisely aligned with evoked versus cross-sectional phenotypes.

Abbreviations: AA, allergic asthma; ACC, aeroallergen challenge chamber; ARC, allergic rhinoconjunctivitis; IgE, immunoglobulin E.

1 | INTRODUCTION

The two-compartments (upper and lower airways)/one-disease paradigm[1,2] is based on the observation that allergic rhinoconjunctivitis (ARC) and allergic asthma (AA) are prevalent, comorbid, environmentally triggered diseases that share mechanistic traits. Common disease triggers of ARC/AA include viral infections and aeroallergens associated with seasonal (eg, mountain cedar) and perennial (eg, house dust mites [HDMs]) ARC.[3,4] Because ARC/AA are environmentally triggered conditions, complex host x environment interactions may determine the level of symptoms evoked following exposure to a disease trigger (evoked severity; Figure 1A, right). The contribution of host factors may be large. Given the ubiquity of and repeated exposures to aeroallergens, and the high prevalence of atopy to aeroallergens in the general population, a conundrum remains: why is it that only a small proportion of atopic individuals progress to develop severe ARC/AA? We posited that most atopic persons preserve mechanisms which enforce minimal responsiveness (resilient) or adaptation to repeated aeroallergen exposures correlating with moderate levels of symptoms; conversely, loss of these mechanisms associates with a maladaptive response that correlates with more-severe disease. We named these three evoked response groupings as the resilient, adaptive, and maladaptive evoked phenotypes, respectively (Figure 1A, right). Conceivably, the mechanistic correlates of ARC/AA may more closely align with how an atopic individual responds to a disease trigger vs. how they differ in resting or constitutive state (Figure 1A, right vs. left, respectively). That is, ARC/AA phenotypes defined by symptom levels in response to exposure to disease triggers in a controlled setting may be a more precise proxy for disease severity and associated mechanisms vs. phenotypes defined by cross-sectional evaluations of non-evoked (constitutive) symptom levels during a resting state.

However, the conventional method for phenotyping persons with ARC/AA is based on cross-sectional comparisons of persons categorized according to non-evoked disease severity (mild vs. moderate vs. severe) and/or biomarker levels (eg, T helper-2 [Th2]-high asthma) recorded in the resting state (ie, baseline; Figure 1A, left). Since the constitutive cross-sectional phenotypes omit the host response to an environmental disease trigger, such phenotypes may group individuals with contrasting evoked phenotypes (Figure 1B). Additionally, cross-sectional phenotyping may be confounded by the extensive variation in the concentration of multiple aeroallergens in

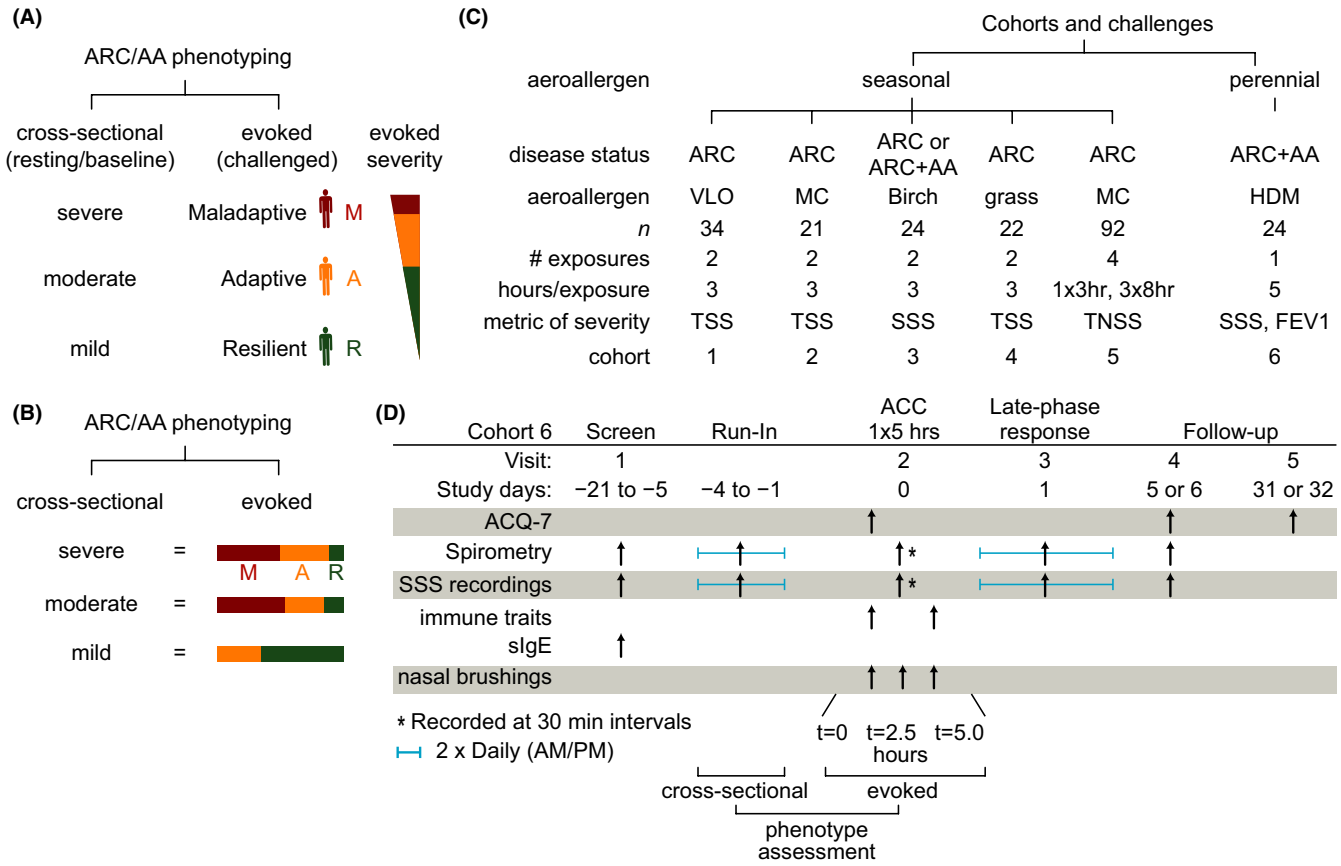


FIGURE 1 Models, cohorts and study design. (A) Cross-sectional vs. evoked phenotypes. (B) Evoked phenotypes by cross-sectional phenotypes. (C) Cohorts studied. (D) HDM+PARC+AA+ (cohort 6) study design and time windows for phenotype assessments. A, adaptive; AA, allergic asthma; ACC, aeroallergen challenge chamber; ACQ-7, asthma control questionnaire-7; ARC, allergic rhinoconjunctivitis; FEV1, forced expiratory volume within one second; HDM, house dust mite; hr, hour; M, maladaptive; MC, mountain cedar; R, resilient; sIgE, house dust mite-specific IgE; SSS, summated symptom scores; t, timepoint; TNSS, total nasal symptom scores; TSS, total symptom scores; VLO, Virginia live oak

the natural environment as well as other environmental factors (eg, dust and temperature) that may influence symptom severity.[5] To mitigate these confounders, we initiated studies to examine evoked ARC/AA phenotypes elicited during exposure to a fixed concentration of an aeroallergen in an aeroallergen challenge chamber (ACC).[6,7] In a recent study,[8] we challenged persons with HDM-associated perennial ARC without physician-diagnosed asthma (henceforth, HDM+PARC+AA−) on four consecutive days with a fixed concentration of HDMs in the ACC. Participants manifested fully or partially restrained vs. unrestrained evoked symptom responsiveness corresponding to mild, moderate, and higher disease severity, respectively. We assigned these patterns of provoked responses to signify the resilient, adaptive, and maladaptive evoked phenotypes, respectively.[8] Those studies also supported the idea that the constitutive cross-sectional phenotypes group persons with different evoked phenotypes[8] (eg, Figure 1B). This grouping masked the true mechanistic correlates of disease severity in HDM+PARC+AA− persons.[8]

To confirm that the distinction between evoked vs. constitutive phenotypes is not spurious and extends beyond HDM+PARC+AA− persons, we undertook a comprehensive evaluation of challenge studies conducted in persons with (i) ARC attributable to seasonal aeroallergens, and (ii) HDM-associated PARC with AA (henceforth HDM+PARC+AA+). To scale duration (3–8 h) and frequency (1–4) of exposures, diversity of aeroallergens (seasonal and perennial), and both elements of the two-compartments/one-disease paradigm (ARC and AA), we examined six ACC challenge cohorts (Table S1). None of the study participants overlapped with those in our recent study[8] and basic features of three of these cohorts have been described previously.[5,9] Because of concerns that exposure to HDMs may provoke a severe asthma attack, the challenge studies in HDM+PARC+AA+ persons represented proof-of-principle studies to determine the feasibility of safely performing challenge studies in persons with AA. Therefore, for caution and careful monitoring, we restricted the (i) number of HDM+PARC+AA+ persons who were simultaneously exposed to HDMs, (ii) challenge to a single 5-h HDM exposure, and (iii) inclusion criteria to persons with mild to moderate HDM+PARC+AA+. For each of the challenge studies, we mitigated the confounding effects of additive environmental influences on ARC/AA severity by performing the ACC studies in time windows when ambient environmental levels of aeroallergens were low in South Texas (May to early December) and chances of winter season-associated (eg, cold air and viral infection) triggers of ARC/AA were not high.

2 | METHODS

The IntegReview Institutional Review Board (Austin, TX) approved studies related to ACC exposures and the University of Texas Health Science Center at San Antonio Institutional Review Board approved the mechanistic aspects of the studies. Cohort characteristics, experimental protocols, disease metrics, and biostatistical/bioinformatic methods are outlined in Tables S1 and S2 and the Appendix S1.

2.1 | Study design

The study had two overall objectives. First, we examined the range of evoked phenotypes elicited following challenges to an array of seasonal aeroallergens, including pollens that are not endemic to the geographic region where these challenge studies were performed (Figure 1C; Table S1). Second, we characterized evoked phenotypes and associated mechanisms in HDM+PARC+AA+ persons challenged with HDM [note: in our previous study, we omitted individuals with physician-diagnosed asthma[8]].

2.2 | Cohorts

Symptom severity metrics were available from six cohorts (Figure 1C). Cohorts 1–5 comprised persons with ARC challenged with seasonal aeroallergens: cohort 1, Virginia live oak (VLO; $n = 34$); cohort 2, mountain cedar (MC; $n = 21$); cohort 3, birch ($n = 24$; 8 with ARC and 16 with both ARC and AA); cohort 4, Timothy grass ($n = 22$), and cohort 5, mountain cedar ($n = 92$). Cohort 6 comprised HDM+PARC+AA+ persons challenged with HDM ($n = 24$; Figure 1C,D; Figure S1A,B; Table S3). To provide context to the findings observed in cohort 6, we compared the level of disease severity evoked in response to HDM exposure in the HDM+PARC+AA+ cohort studied herein to that in a cohort of HDM+PARC+AA− individuals ($n = 23$) studied previously.[8]

2.3 | ACC operations

The ACC facility, operations, as well as methods for aeroallergen dispersal and monitoring are described (Note S1). The exposure regimens are depicted (Figure 1C,D) and the challenge protocols were synonymous among each other and with our previous studies.[8–14] The only exception is for cohort 3, as this study was performed during the COVID-19 pandemic; the ACC was modified to ensure safety (Note S1). Pollen concentrations used for Timothy grass and birch were the same as previously published by other chamber facilities to elicit symptoms comparable to those experienced during natural seasonal exposure[15,16] (Table S1). Pollen concentrations for mountain cedar and Virginia live oak were determined in our ACC through a series of trials utilizing increasing concentrations until adequate seasonal-level symptoms were generated[5] (Table S1).

2.4 | Metrics of symptom severity and evoked phenotype definition

Metrics of symptom severity available for analysis were the total nasal symptom scores [TNSS], total ocular symptom scores [TOSS], and total asthma symptom scores [TASS] (Figure S1C; Table S4). Total symptom scores (TSS) summate TNSS and TOSS, and summated symptom scores (SSS) summate TSS and TASS. Adhering to conventional practices,[17–19] instantaneous and reflective symptom scores recorded by the participants are indicated by the prefixes i and r, respectively. The prefix i before a metric (eg, iSSS) indicates

that the score was recorded instantaneously at defined intervals in the ACC and represents the symptoms experienced over the past 10–15 min. In contrast, the prefix r (eg, rSSS) indicates that the score was recorded by the participant in their natural environment outside the ACC and represents a reflective recording of the symptoms experienced over a 12-h period (AM/PM). Symptom metrics available for derivation of evoked phenotypes were: iTSS in cohorts 1, 2, and 4; iSSS in cohort 3; iTNSS in cohort 5, and iSSS in cohort 6. Participants who manifested fully restrained, partially restrained, or unrestrained evoked symptom responsiveness were classified as having resilient, adaptive, or maladaptive phenotypes, respectively.

2.5 | Evoked phenotypes in cohorts 1–5: seasonal aeroallergens

Symptom metrics recorded at 30-min intervals during the ACC exposures were used for clustering analyses (unsupervised hierarchical clustering with Ward's D linkage and Manhattan distances; Appendix S1). The assignment of evoked phenotypes was based on the pattern of the trajectories of symptom severity metrics corresponding to the clusters identified. In cohorts 1–4, assignment of evoked phenotypes was based on the 14 symptom metric recordings obtained during exposure days 1 and 2. Evoked phenotype assignment in cohort 5 was based on the 7 symptom metric recordings obtained during exposure day 1. This difference in evoked phenotype assignment in cohort 5 vs. cohorts 1–4 was based on the study design. In cohort 5, there was a 3-day interval between the initial 3-h aeroallergen exposure (day 1) and subsequent exposures (days 2, 3, and 4) which were longer (8 h/day) and occurred on consecutive days (Figure 1C). In contrast, aeroallergen exposures in cohorts 1–4 were for 3 h/day on two consecutive days (Figure 1C). Nasal sampling was not performed in cohorts 1–5. A meta-analysis was performed with symptom metrics shared by cohorts 1, 2 and 4. In this meta-analysis, the associations of evoked phenotypes with IgE specific to the challenged aeroallergen were also determined through ImmunoCAP assay performed by ThermoFisher.

2.6 | Cohort 6: HDM+PARC+AA+ persons challenged with HDMs

2.6.1 | Study design

We prescreened 44 persons with HDM+PARC+AA+ with intermittent mild to moderate asthma; 27 (61%) demonstrated forced expiratory volume within one second (FEV1) reversibility ≥12% and HDM skin prick test positivity (Figure S1A; Table S3). Of these 27 subjects, three were disqualified based on predefined criteria (Figure S1A, Table S3). Persons recorded symptom severity and FEV1 before, during, and after the ACC challenge (Figure 1D). The study comprised five phases/visits (Appendix S1): screening (visit 1: days −21 to −5), run-in (days −4 to −1), ACC exposure (visit 2: day 0), late-phase response visit (visit 3: day +1), and two follow-up visits 1 and ~5 weeks post-ACC exposure (Figure 1D). Visit 2 comprised a one day, 5-h exposure to HDM powder (Figure 1D; Figure S1B); the HDM powder used was from the same batch we used previously[8,10]. Participants recorded symptom severity metrics and FEV1 during the run-in phase (AM and PM), ACC exposure (at 30-min intervals), and for 4 days post-ACC exposure (AM and PM) that included during the late-phase response visit. Asthma control questionnaire-7 (ACQ-7) scores (a metric of asthma control)[20] were obtained from participants at visits 2, 4, and 5.

2.6.2 | Phenotype definitions

Evoked vs. constitutive cross-sectional phenotypes were derived (Figure 1D). Tertiles of non-evoked symptom metrics self-recorded in the participant's natural environment during the run-in phase (days −4 to −1) defined the constitutive cross-sectional phenotypes. In contrast, clustering analysis of evoked symptom metrics recorded in the ACC (visit 2) was used to define the evoked phenotypes.

2.6.3 | Peripheral blood measures

Peripheral blood was sampled before and after the ACC challenge for assessment of immune cell traits (including leukocyte blood counts) and levels of serum HDM-specific IgE (sIgE) (Figure 1D), using methods described previously.[8,10] Eighty-six immune cell traits were analyzed (antibody panels in Table S5). The cutoffs for peripheral blood eosinophil, neutrophil, and sIgE levels evaluated as categorical traits were: eosinophil^{hi}: 150 cells/μL, neutrophil^{hi}: 1×10^6 cells/mL, and sIgE^{hi}: 0.35 kU/L. The rationale for the cutoffs is outlined (Appendix S1).

2.6.4 | Clustering approaches

Unsupervised hierarchical clustering with Ward's D linkage and Manhattan distances were used to derive evoked phenotypes with 11 iSSS measures recorded at 30-min intervals in the ACC. Unsupervised clustering was performed with baseline peripheral blood CD8+ T-cell counts and CD4:CD8 T-cell ratio values using Euclidean distances and Ward's D linkage; these clusters were defined as the CD8-CD4 balance clusters. The association of peripheral blood T-cell immune traits with evoked phenotypes was examined by performing unsupervised clustering of significant T-cell traits using Ward's D linkage and Euclidean distances as well as supervised clustering of participants by evoked phenotype.

2.6.5 | Nasal cell gene expression profiling

Nasal brushings were obtained before, in the middle, and after the ACC challenge ($t = 0$, 2.5 and 5 h, respectively, in visit 2; Figure 1D). Gene expression profiling by RNA-Seq was performed on cells obtained by nasal brushings as previously described[8,10] (Appendix S1). Expression of genes relevant to AA was monitored via the asthma^{up} 18-gene signature (Appendix S1).

2.7 | Statistical analyses

Detailed statistical analyses and approaches are outlined in the figure legends and Table S2. Briefly, statistical analyses of clinical and immunophenotyping data included Fisher's exact test, *z*-transformation, linear regression, likelihood ratio test (LRT), and analysis of variance (ANOVA). For analysis of repeated measurements from the same study participant, a linear generalized estimating equation (GEE) model with an exchangeable correlation structure was used; if data were longitudinal, the model was adjusted by time as a factorial variable. To quantify aggregated (overall) symptom severity, area under the curve of symptom metrics were calculated (eg, iTNSS-AUC, iTSS-AUC, iSSS-AUC, and FEV1-AUC). An FDR <0.05 was used to identify significant immune cell traits associated with evoked phenotypes. For longitudinal analysis of gene expression data, GEE-generalized linear model (GLM) models based on the normal distribution (*z*-scores) and gamma distribution (single genes) were used. Models were adjusted by time and had an autoregressive (1) correlation structure; significance was tested using ANOVA. For cross-sectional comparisons, negative binomial GLM was implemented in DESeq and a linear model (z-scores) was used; statistical significance was tested by LRT.

3 | RESULTS

3.1 | Adverse events

There were no serious adverse events (Table S1). The only adverse event during a pollen challenge (cohort 1) was unilateral ocular redness and edema because of direct inoculation, as the participant rubbed the eye by hand rather than using a tissue.

3.2 | Evoked phenotypes in response to seasonal aeroallergens: cohorts 1–5

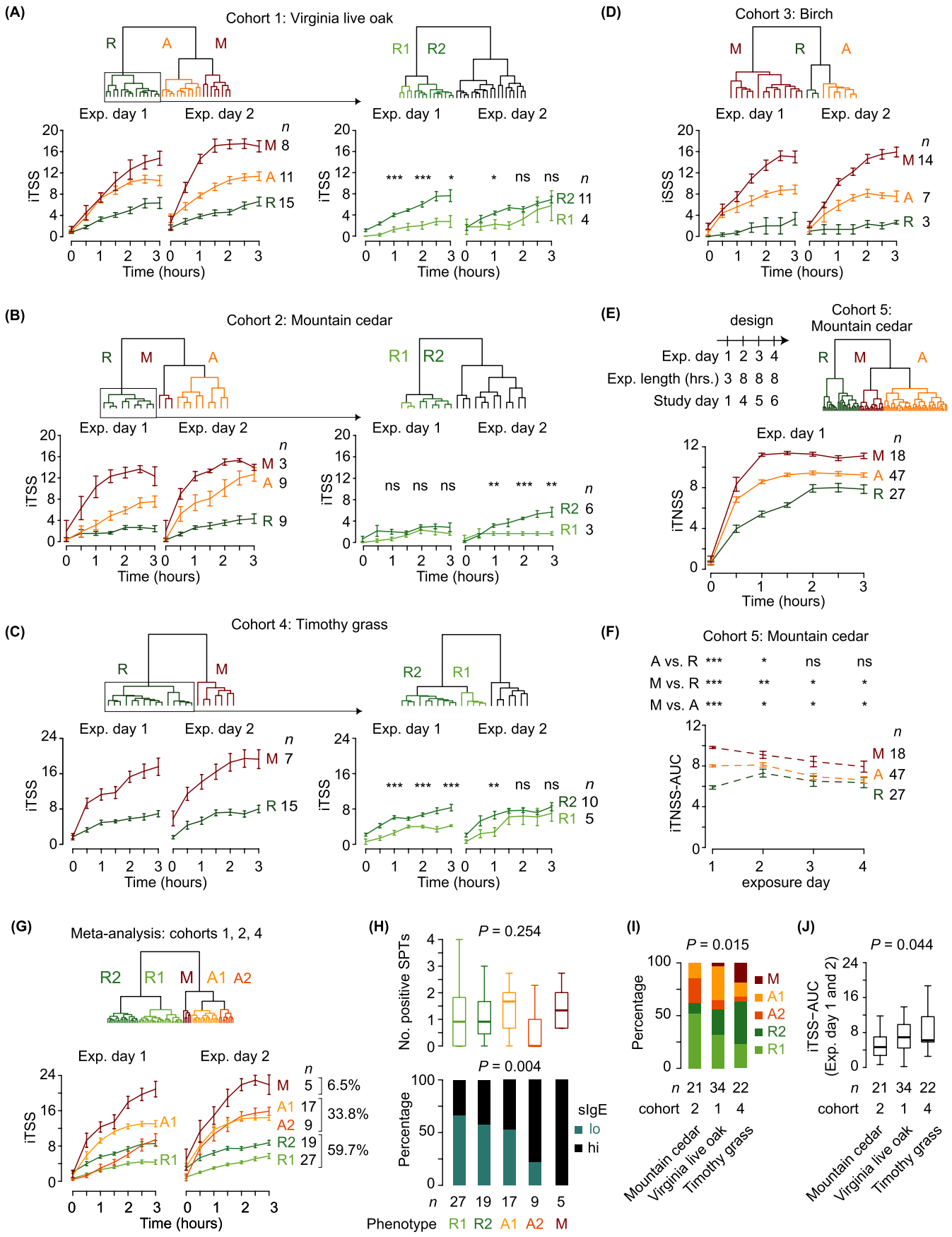
The symptom responses evoked during two shorter (3 h/each; cohorts 1–4) as well as prolonged and repeated (cohort 5) exposures to seasonal aeroallergens were analyzed (Figure 1C; Figure 2). At baseline in the ACC (ie, prior to initiating aeroallergen dispersal), symptom metric recordings approximated zero in cohorts 1–5. During the aeroallergen exposures, three symptom severity clusters corresponding to the resilient, adaptive, and maladaptive phenotypes were observed in cohorts 1, 2 and 3 (Figure 2A,B,D). In cohort 4, only two clusters were observed (Figure 2C).

In cohort 5, three clusters were observed during the initial 3-h exposure to mountain cedar (Figure 2E, top right). In the subsequent 8-h exposures/day on three consecutive days, two temporal patterns were observed (Figure S2). First, during exposure day 2, iTNSS trajectories differed by phenotype (Figure S2). Second, during exposure days 3 and 4, iTNSS trajectories of the adaptive and resilient phenotypes approximated and were lower than those of the maladaptive phenotype (Figure S2). The hierarchy of the overall iTNSS (iTNSS-AUC) during exposure days 1 and 2 was maladaptive > adaptive > resilient phenotype, whereas on exposure days 3 and 4 it was maladaptive > adaptive ~ resilient phenotype (Figure 2F). Thus, the maladaptive phenotype associated with the highest symptom responsiveness during prolonged and repeated exposures with mountain cedar. However, suggesting adaptation, the adaptive phenotype resembled the resilient phenotype by exposure day 4.

3.3 | Post hoc analysis of seasonal aeroallergen cohorts

Despite the generalizability of the concept of evoked phenotypes across 5 cohorts, two exceptions were noted. First, contrary to the other cohorts, only two clusters suggestive of the maladaptive and resilient phenotypes were observed in persons challenged with Timothy grass, comprising 32% and 68% of the cohort, respectively (Figure 2C). We performed post hoc analysis to mitigate the possibility that the failure to observe 3 clusters was attributable to confounding by conflation within the resilient phenotype, that is, the resilient phenotype was a grouping of individuals with the resilient and adaptive phenotypes. We subdivided the resilient phenotype and compared the two subclusters (resilient-1 and resilient-2, comprising 5 and 10 persons, respectively; Figure 2C, right). The iTSS trajectories of these two subclusters did not identify an adaptive-like group within the resilient phenotype. Similar post hoc analysis in cohorts 1 and 2 also did not identify an adaptive-like group within the resilient phenotype (Figure 2A,B, right). Thus, results of these post hoc analyses highlight the fidelity of our clustering approach for

FIGURE 2 Evoked phenotypes during exposures to seasonal aeroallergens in an aeroallergen challenge chamber. (A–D) *Top*: Instantaneous total symptom score (iTSS) or summated symptom score (iSSS) clusters. *Bottom*: Mean (SEM) iTSS or iSSS at 30-min intervals during exposure (Exp.) days 1 and 2 by phenotypes (resilient [R], adaptive [A], maladaptive [M]). Cohort and exposures are indicated. (A–C, right) Post hoc analysis performed on the resilient (R) phenotype cluster; resilient-subclusters are R1 and R2. (E) *Top left*: Study design. *Top right*: Instantaneous total nasal symptom score (iTNSS) clusters. *Bottom*: Mean (SEM) iTNSS at 30-min intervals during exposure (Exp.) day 1 by phenotypes (R, A, M). (F) Mean (SEM) iTNSS-area under the curve (iTNSS-AUC) during exposure days 1, 2, 3, and 4 in cohort 5 participants by R, A, and M phenotypes. (G–J) Meta-analysis using iTSS data from cohorts 1, 2 and 4. (G) *Top*: Clusters. *Bottom*: Mean (SEM) iTSS at 30-min intervals during exposure (Exp.) days 1 and 2 by clusters. The resilient and adaptive subclusters are indicated by suffixes 1 and 2. (H) *Top*: Boxplots (MIQ) of the number of positive skin prick tests (SPTs), adjusted by cohort, by evoked phenotypes (panel G). *p*, by negative binomial GLM with likelihood ratio test (LRT) (adjusted by cohort). *Bottom*: Proportion of persons with $sIgE^{lo}$ or $sIgE^{hi}$ by meta-analysis evoked phenotypes (panel G). *p*, by logistic regression with LRT (adjusted by cohort). Cutoff for $sIgE^{hi}$ vs. $sIgE^{lo}$: 0.35 kU/L (Appendix S1). (I) Proportion of the indicated clusters in cohorts 2 (mountain cedar), 1 (Virginia live oak), or 4 (Timothy grass). (J) Boxplots of mean iTSS-AUC of exposure (Exp.) days 1 and 2 by cohort. *p*, by LRT or Fisher's exact test; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, ns, non-significant


(A)
Cohort 1: Virginia live oak
R A M
R1 R2
Exp. day 1 Exp. day 2
iTSS
Time (hours)
n
M 8
A 11
R 15
*** *** * * ns ns n
R2 11
R1 4
(B)
Cohort 2: Mountain cedar
R M A
R1 R2
M 3
A 9
R 9
ns ns ns ** *** ** n
R2 6
R1 3
(C)
Cohort 4: Timothy grass
R M
R2 R1
M 7
R 15
*** *** *** ** ns ns n
R2 10
R1 5
(D)
Cohort 3: Birch
M R A
iSSS
M 14
A 7
R 3
(E)
design
Exp. day 1 2 3 4
Exp. length (hrs.) 3 8 8 8
Study day 1 4 5 6
Cohort 5: Mountain cedar
R M A
iTNSS
M 18
A 47
R 27
(F)
Cohort 5: Mountain cedar
A vs. R *** * ns ns
M vs. R *** ** * *
M vs. A *** * * *
iTNSS-AUC
exposure day
(G)
Meta-analysis: cohorts 1, 2, 4
R2 R1 M A1 A2
M 5 6.5%
A1 17 33.8%
A2 9
R2 19 59.7%
R1 27
(H)
P = 0.254
No. positive SPTs
P = 0.004
Percentage
sIgE
lo
hi
Phenotype R1 R2 A1 A2 M
(I)
P = 0.015
Percentage
M
A1
A2
R2
R1
cohort 2 1 4
Mountain cedar
Virginia live oak
Timothy grass
(J)
P = 0.044
iTSS-AUC (Exp. day 1 and 2)
cohort 2 1 4
Mountain cedar
Virginia live oak
Timothy grass

assignment of phenotypes and suggest that the 2-cluster response observed in participants challenged with Timothy grass was unlikely to be spurious.

Second, approximately 58% of birch-sensitive persons (cohort 3) clustered to the maladaptive phenotype. In contrast, the proportions of individuals with the maladaptive phenotype in cohorts 1, 2, 4, and 5 were substantially lower (24%, 14%, 32%, and 20%). Timothy grass and birch pollens are not endemic to the geographic region where the challenge studies were performed. Exposure to a non-endemic vs. endemic (eg, mountain cedar) aeroallergen could potentially evoke different levels of responses. Correspondingly, the maladaptive phenotype was more frequent in cohorts 3 and 4 (58% and 32%, respectively; birch and Timothy grass), comprising ARC individuals challenged with non-endemic pollens.

3.4 | Meta-analysis of seasonal aeroallergen challenge studies

To define the range of evoked phenotypes with a larger sample size, we performed a meta-analysis of three cohorts with the iTSS metric (cohorts, 1, 2, and 4; Figure 2G). This meta-analysis revealed that some persons showed the same (maladaptive cluster and adaptive subcluster-1) or higher (adaptive subcluster-2) symptom responsiveness on repeat challenge, whereas others showed resistance to increases in symptoms on a repeat challenge (resilient subcluster-1 and resilient subcluster-2; Figure 2G). This meta-analysis reinforced that despite the ubiquity of seasonal aeroallergens, after exposure to these disease triggers in a controlled setting, most persons manifest a resilient followed by the adaptive evoked phenotype (59.7% and 33.8%, respectively). Thus, only a minority manifest the maladaptive phenotype, signifying more-severe disease. The level of polysensitization (number of positive skin prick tests) did not statistically differ (after adjusting for cohort) among the evoked phenotypes (Figure 2H, top; Note S2). However, a significant association between seasonal aeroallergen-specific IgE levels and the evoked phenotypes was evident (Figure 2H, bottom). Higher levels of seasonal aeroallergen-specific IgE were overrepresented in persons with the adaptive subcluster-2 (A2) and the maladaptive phenotype (Figure 2H, bottom).

Representation of the resilient and adaptive phenotypes was highest in the mountain cedar and least in the Timothy grass cohorts (Figure 2I). The hierarchy of the overall symptom responsiveness was Timothy grass > Virginia live oak > mountain cedar (Figure 2J). Hence, resilience and adaptation to seasonal aeroallergens appeared to be more prevalent in response to endemic (mountain cedar and oak) versus non-endemic (Timothy grass) pollens.

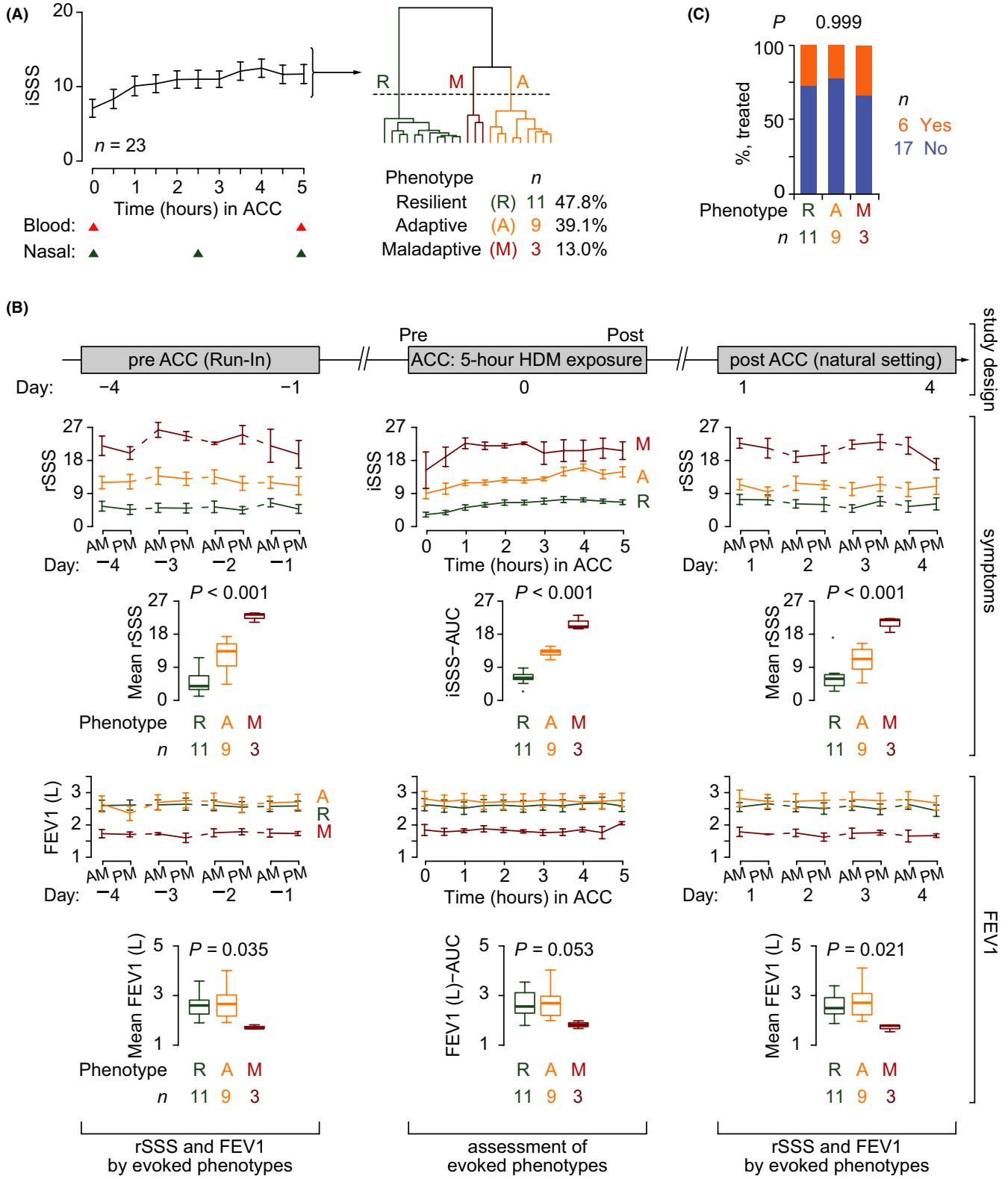
3.5 | Cohort 6. HDM+PARC+AA+ phenotypes

Twenty-three HDM+PARC+AA+ participants completed the 5-h exposure to HDMs (Figure S1A,B); one participant was removed from the ACC based on protocol specifications (a decrease in FEV1 of ≥12% experienced on two occasions during the ACC that was not reversible with nebulized bronchodilators; Appendix S1). While the mean iSSS increased in response to HDM exposure (Figure 3A; left; $p < .001$), unsupervised hierarchical clustering identified three iSSS clusters (Figure 3A; right). The resilient phenotype tracked individuals with lower iSSS at baseline (pre-exposure, $t = 0$) and minimal responsiveness to HDM exposure [Figure 3B (iSSS); *middle* column-top row; green]. The adaptive and maladaptive phenotypes were characterized by intermediate and higher iSSS at baseline, respectively, as well as intermediate and higher responsiveness to HDM exposure [Figure 3B (iSSS); *middle* column-top row; orange and red, respectively]. The iSSS-AUC metric, capturing the overall symptom severity during the ACC, showed a hierarchical pattern: maladaptive > adaptive > resilient phenotype (Figure 3B, *middle* column-second row).

Median age (interquartile range) and body mass index of the overall cohort were 44 (36–51) years and 33.6 (27.2–39.0), respectively; 65.2 percent were women (Table S6). Phenotypes did not differ significantly by demographics or other characteristics (race, income, or time spent outdoors; Table S6) or by wheal reactivity to skin prick test for various aeroallergens (Table S7). These findings suggest that the phenotypes related to responsiveness to HDM exposure rather than demographic and skin prick test characteristics.

In clinical practice, the correlation between FEV1 and asthma symptom scores is low[21]; correspondingly, aside from a weak correlation with iTASS ($r = -.31$, $p = .157$), FEV1 did not correlate with symptom severity metrics in the ACC (Figure S3). The proportion of individuals who received nebulized bronchodilator treatment for a drop in FEV1 did not differ by phenotype (Figure 3C). Moreover, the hierarchy of symptom severity (iSSS)

FIGURE 3 Evoked phenotypes in persons with house dust mite (HDM)-associated perennial allergic rhinoconjunctivitis with asthma (HDM+PARC+AA+) identified during exposure to HDMs in an aeroallergen challenge chamber (ACC). (A) *Left*: Mean (SEM) instantaneous summated symptom scores (iSSS) at 30-min intervals in HDM+PARC+AA+ participants during a 5-h exposure to HDMs; triangles indicate sampling timepoints. *Right*: Clusters and phenotypes (resilient [R], adaptive [A], maladaptive [M]). (B) *Top*: Study design schema. All data are based on the symptom metrics and FEV1 according to the evoked phenotypes that were identified during the HDM exposure. *Bottom* (plots): *First row*: Mean (SEM) reflective summated symptom score (rSSS) and instantaneous SSS (iSSS) at the indicated timepoints by HDM+PARC+AA+ phenotypes before (run-in), during, and after (natural setting) the ACC challenge. *Second row*: Mean rSSS (SEM) in the 4-day run-in and natural settings, and iSSS-area under the curve (AUC) in the ACC. *Third row*: Mean (SEM) FEV1 at the indicated timepoints by HDM+PARC+AA+ phenotypes before (run-in), during, and after (natural setting) the ACC challenge. *Fourth row*: Mean FEV1 (SEM) in the 4-day run-in and natural setting, and FEV1-AUC in the ACC. *p*, by likelihood ratio test (LRT). (C) Proportion of HDM+PARC+AA+ participants with the R, A, or M phenotypes who required treatment during the ACC due to a decline in FEV1 ≥12% (Appendix S1). *p*, by Fisher's exact test. AA, allergic asthma; FEV1, forced expiratory volume within one second; L, liters.; PARC, perennial allergic rhinoconjunctivitis



and FEV1 by evoked phenotype differed. While iSSS showed a hierarchical association by phenotype, FEV1 did not [Figure 3B (FEV1); *middle* column first and second vs. third and fourth rows]. During the ACC exposure, FEV1 was lowest in the maladaptive phenotype but similar in the resilient and adaptive phenotypes (resilient ~ adaptive > maladaptive).

We next examined whether the three evoked phenotype groupings were an accurate descriptor of symptom severity and FEV1 recoded by participants in their natural settings before (run-in; pre-ACC) and after (post-ACC) the challenge. The hierarchy of the rSSS and FEV1 recorded before and after the ACC according to the evoked phenotypes was similar to the hierarchy of iSSS and FEV1

recorded in the ACC (Figure 3B, left and right columns). Hence, we observed that the distinct hierarchies of symptom severity (maladaptive > adaptive > resilient) and FEV1 (resilient ~ adaptive > maladaptive) observed in the ACC were preserved in the natural settings (pre- and post-ACC) (Figure 3B).

Examination of the individual components of iSSS (iTSS and iTASS; Figure 4A, Figure S4A) revealed that the hierarchy of iTSS by phenotype was maladaptive >adaptive > resilient, whereas by iTASS it was maladaptive > adaptive ~ resilient (Figure 4A; Figure S4A). These findings suggest that, despite the two-compartments/one-disease paradigm, the hierarchical relationship of the metrics of lower (TASS and FEV1) and upper (TSS) airways differs by evoked phenotypes (Figure 4B). Thus, the adaptive and resilient phenotypes were similar in that they shared comparable FEV1 values, whereas the iTSS and iTASS of the adaptive phenotype were intermediate between the resilient and maladaptive phenotypes.

3.6 | Cohort 6. Confounding with constitutive cross-sectional phenotyping

To examine whether symptom severity recorded in the natural setting before ACC challenges (constitutive cross-sectional phenotypes) accurately predicted the evoked phenotypes, we stratified HDM+PARC+AA+ individuals according to tertiles of their average-rSSS scores recorded for 4 days during the run-in phase (Figure 1D). Cross-sectional phenotypes defined by the run-in tertiles misclassified the evoked phenotypes (Figure 4C). While the first run-in rSSS tertile was overrepresented in individuals with the resilient phenotype, some individuals with the adaptive phenotype were misclassified to this tertile (Figure 4C). The extent of misclassification was greater in the other two tertiles, as the second run-in rSSS tertile represented a grouping of the resilient and adaptive phenotypes in nearly equal proportions, whereas the third rSSS tertile was a grouping of the adaptive and maladaptive phenotypes in nearly equal proportions (Figure 4C). Four findings indicate that because of this grouping, the rSSS run-in tertiles (cross-sectional phenotypes) were imprecise predictors of symptom responsiveness or FEV1 recorded in the ACC (compare Figure 4D vs. 3B; Figure S4B,C; summarized in Figure 4B,E).

First, the level of symptom responsiveness during the ACC exposure associated with the first and second run-in tertiles in the ACC were similar, whereas the evoked phenotypes associated with significantly different levels of responsiveness. Second, the symptom responsiveness during the ACC exposure associated with the third run-in tertile was less than that associated with the maladaptive phenotype. Third, an average run-in rSSS ≥19.31 or baseline (t = 0) FEV1 ≤ 2.84 were cutoffs that distinguished persons with the maladaptive vs. the resilient and adaptive phenotypes (Figures 3B and 4E); however, cutoffs that distinguished the resilient vs. adaptive phenotypes were not evident. However, identifying the maladaptive phenotype would not have been possible *a priori*, as these cutoffs were identified after HDM challenge. Fourth, the run-in tertiles associated with similar FEV1 in the ACC, whereas FEV1 differed by evoked phenotypes. Thus, there was discordance in disease severity metrics and FEV1 associated with the evoked and cross-sectional phenotypes (Figure 4B). Correspondingly, the evoked phenotypes explained 92% of the variability in the overall symptoms experienced in the ACC, whereas the cross-sectional phenotypes explained 52% of the variability. Moreover, rSSS run-in tertiles also misclassified ACQ-7 scores (Figure 4F). ACQ-7 scores assessed at visits 2, 4, and 5 in HDM+PARC+AA+ individuals were higher in the maladaptive phenotype, but similar in the adaptive and resilient phenotypes (Figure 4F, leftmost; Figure S5). However, ACQ-7 scores stratified according to the run-in tertiles were not significant (Figure 4F, second from left; Figure S5).

Further corroborating the precision and reproducibility of the phenotyping in the ACC, we observed a high level of congruence in the evoked phenotypes in two separate HDM+PARC+ cohorts challenged with HDMs: HDM+PARC+AA+ persons (cohort 6) and HDM+PARC+AA− persons reported previously[8] (Figure 4G). The representation of the evoked phenotypes in both cohorts was similar (Figure 4G, left). iTSS was a shared symptom severity metric recorded in the two cohorts. The level of this metric in the overall cohort as well as by phenotype was similar (Figure 4G, right). Thus, HDM exposures elicited a rather stereotypical response in HDM+PARC+ persons, irrespective of their AA status. Because the cross-sectional phenotypes are a combination of evoked phenotypes reported herein (Figure 4C) and previously,[8] use of the run-in tertiles would have masked this stereotypical evoked response.

3.7 | Cohort 6. Conventional laboratory measures and symptom severity

Eosinophils, neutrophils, and IgE have established roles in ARC/AA pathogenesis.[22] We previously found that the balance between CD4+ and CD8+ T-cells changed during HDM challenges and associated with disease severity.[8,10] For these reasons, we examined the associations of these laboratory measures with disease severity and evoked phenotypes in cohort 6 (HDM+PARC+AA+). Unsupervised hierarchical clustering of the baseline (t=0; Figure 1D) CD8+ T-cell count and the CD4:CD8 ratio identified two clusters designated as cluster I [$CD8^{lo}$-$CD4^{lo}$] vs. II [$CD8^{hi}$-$CD4^{hi}$] (termed hereafter as CD8-$CD4^{lo}$ and CD8-$CD4^{hi}$, respectively; Figure 5A). CD8-$CD4^{hi}$ compared with CD8-$CD4^{lo}$ was characterized by higher CD8+ and CD4+ T-cell counts as well as a lower CD4:CD8 ratio (Figure 5A). The CD8-CD4 balance clusters were asymmetrically distributed across the phenotypes, as CD8-$CD4^{hi}$ was underrepresented in persons with the resilient phenotype and overrepresented in those with the adaptive and maladaptive phenotypes (Figure 5B). CD8-$CD4^{hi}$ associated with higher iSSS at t = 0 and during HDM exposure (Figure 5C, leftmost; Figure 5D, middle; Figure S6A) as well as with higher rSSS during the run-in and post-ACC phases of the study (Figure 5D).

We next examined whether higher vs lower baseline (t = 0) values of eosinophils, neutrophils, and sIgE were asymmetrically distributed across the evoked phenotypes. $Eosinophil^{hi}$ was overrepresented in

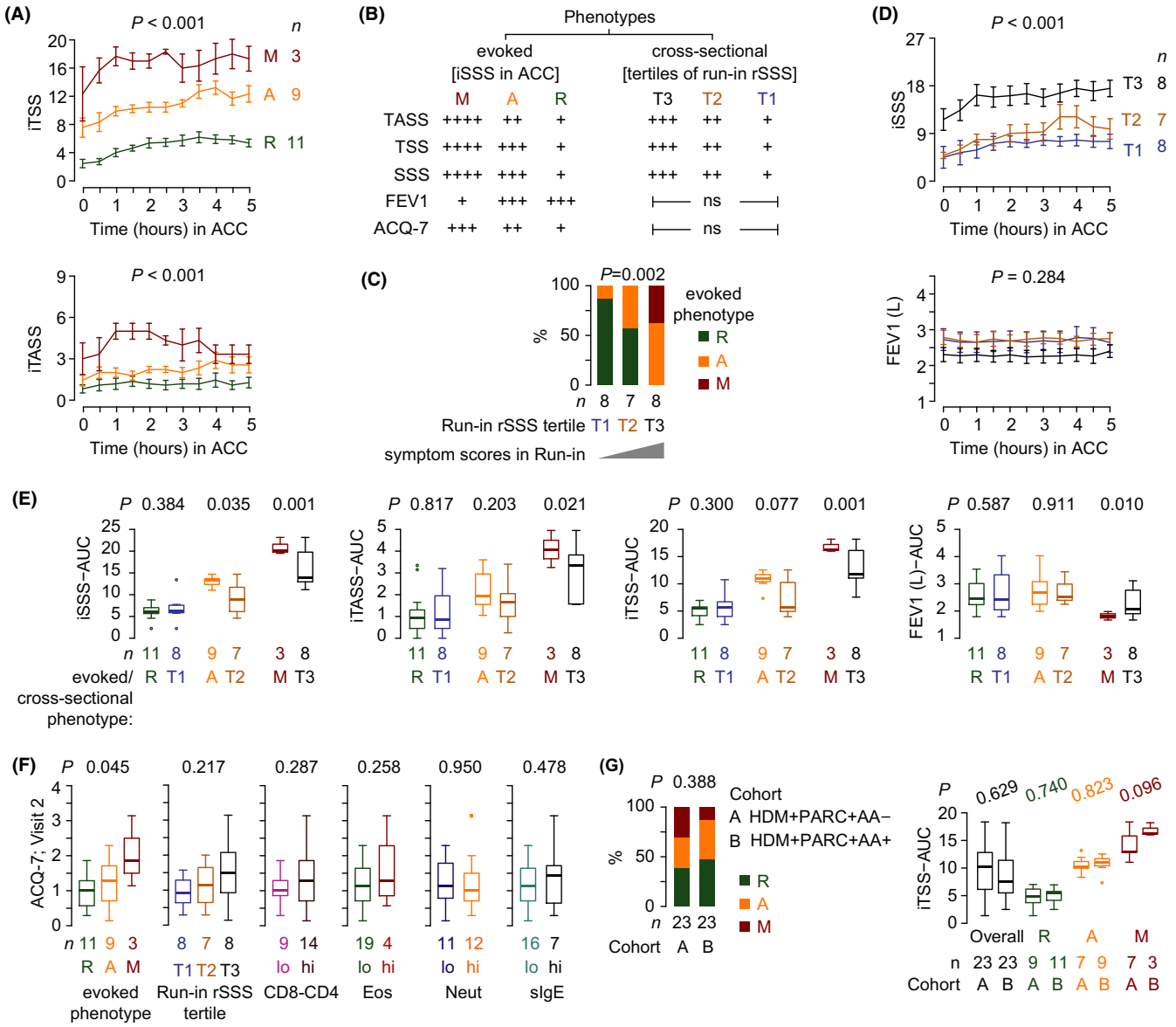


FIGURE 4 Symptom severity and adequacy of asthma control according to evoked versus cross-sectional phenotypes in HDM+PARC+AA+ persons exposed to house dust mites (HDM) in an aeroallergen challenge chamber (ACC). (A) Mean (SEM) (*top*) instantaneous total symptom score (iTSS) and (*bottom*) instantaneous total asthma symptom score (iTASS) at the indicated timepoints in the ACC by phenotypes (resilient [R], adaptive [A], maladaptive [M]). *p*, by likelihood ratio test (LRT). (B) Schema of level of symptom severity, forced expiratory volume within one second (FEV1), and asthma control questionnaire (ACQ)-7 score by evoked phenotypes (R, A, M) and cross-sectional phenotypes [defined by tertiles (T) of run-in reflective summated symptom scores (rSSS); T3 is the tertile with the upper-third values of the average-rSSS]. ns, non-significant. (C) Proportion of evoked phenotypes represented in cross-sectional phenotypes. *p*, by Fisher's exact test. (D) Mean (SEM) (*top*) instantaneous SSS (iSSS) and (*bottom*) FEV1 at the indicated timepoints in the ACC according to run-in rSSS tertiles. *p*, by LRT. T, tertile. (E) Boxplots of (*left to right*) iSSS-area under the curve (AUC), iTASS-AUC, iTSS-AUC, and FEV1 (L)-AUC in the ACC by evoked phenotypes and cross-sectional phenotypes. *p*, by GEE model with an exchangeable correlation structure. L, liters. (F) ACQ-7 scores at visit 2 stratified by (*left to right*) evoked phenotypes, cross-sectional phenotypes, CD8-CD4 balance clusters (described in Figure 5A), and eosinophil (Eos), neutrophil (Neut), and HDM-specific IgE (sIgE) strata. Strata cutoffs in Appendix S1. *p*, by LRT. (G) *Left*: Proportion of HDM+PARC+AA− (Ref.[8]) and HDM+PARC+AA+ (cohort 6) persons with the evoked phenotypes (R, A, and M). *p*, by Fisher's exact test. *Right*: Mean iTSS-AUC in the ACC in HDM+PARC+AA− (Ref.[8]) and HDM+PARC+AA+ (cohort 6) persons overall and by R, A, and M phenotypes. *p*, by LRT. AA, allergic asthma; Hi, higher; Lo, lower; PARC, perennial allergic rhinoconjunctivitis

the maladaptive phenotype, whereas the proportion with eosinophil^{hi} was similarly low in persons with the adaptive and resilient phenotypes (Figure 5B). In contrast, both the lowest and highest run-in tertiles (Figure 4C) associated with eosinophil^{hi} (Figure S7), suggesting that eosinophil levels may more closely align with the evoked vs. cross-sectional phenotypes. Eosinophil^{hi} associated with higher symptom severity at $t = 0$ in the ACC (Figure 5C, Figure S6B). However, during HDM challenge, the differences in symptom severity by eosinophil strata did not achieve statistical significance (Figure 5C,E).

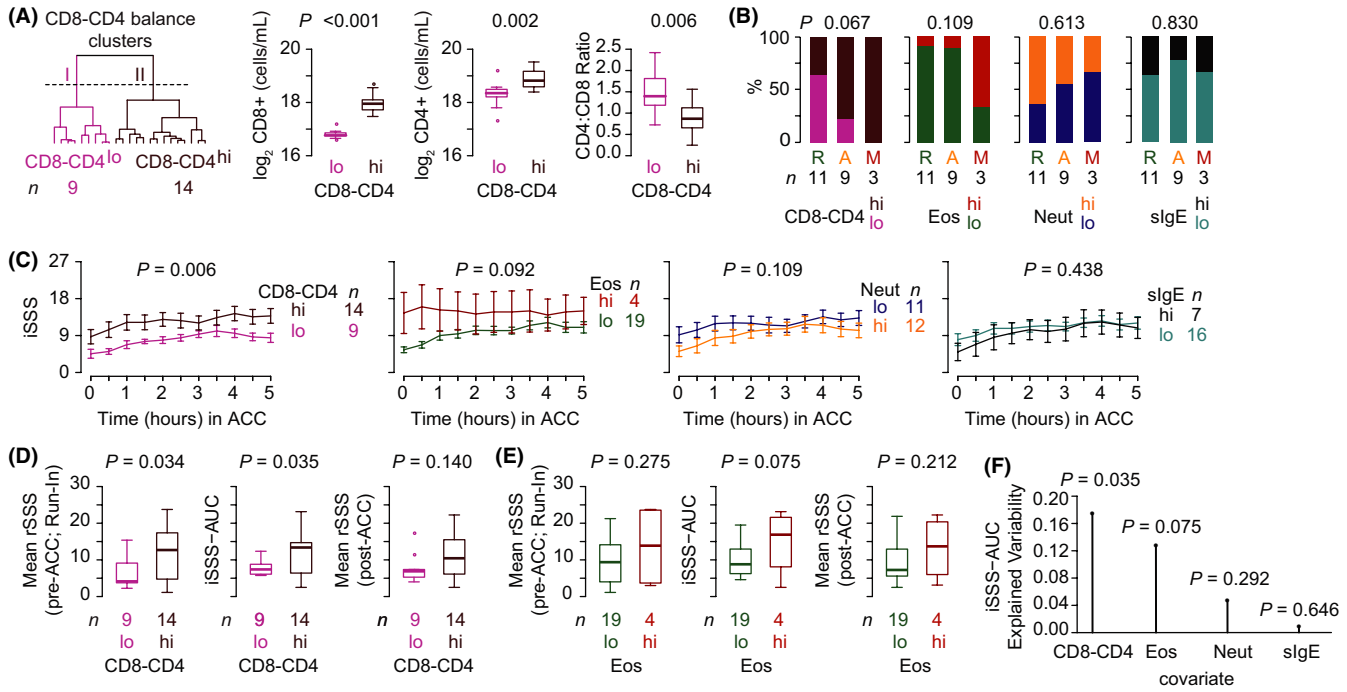


FIGURE 5 Cellular traits associated with symptom severity and evoked phenotypes in HDM+PARC+AA+ persons exposed to house dust mites (HDMs) in an aeroallergen challenge chamber (ACC). (A) *Left*: Two CD8-CD4 balance clusters identified by unsupervised hierarchical clustering of baseline (timepoint $t = 0$ in ACC exposure) CD8+ T-cell counts and CD4:CD8 T-cell ratio levels. *Right*: Baseline CD8+ and CD4+ T-cell counts and CD4:CD8 T-cell ratio levels by CD8-CD4 balance cluster I (CD8-CD4^{lo}) and cluster II (CD8-CD4^{hi}). Y-axes depicted as exponentiation of $\log_2$ scale. *p*, by linear model with likelihood ratio test (LRT) using $\log_2$-transformed values. Hi, higher; Lo, lower. (B) Proportion of evoked phenotypes (resilient [R], adaptive [A], maladaptive [M]) with the indicated peripheral blood trait strata. *p*, by Fisher's exact test. (C) Mean (SEM) instantaneous summated symptom scores (iSSS) during the ACC exposure by (*left to right)* CD8-CD4 balance clusters (CD8-CD4), eosinophil (Eos), neutrophil (Neut), and house dust mite-specific IgE (sIgE) strata. Strata cutoffs in Appendix S1. *p*, by linear GEE model with an autoregressive 1 correlation structure and adjusted by time (in minutes). (D–E) Boxplots of mean reflective SSS (rSSS) in the 4-day run-in and post-ACC phases, and iSSS-area under the curve (AUC) in the ACC in HDM+PARC+AA+ participants stratified by (D) CD8-CD4 balance clusters, and (E) Eos strata. *p*, by linear model with LRT. (F) Coefficient of determination (r^2) with iSSS-AUC as the dependent variable and CD8-CD4$^{hi/lo}$, Eos$^{hi/lo}$, Neut$^{hi/lo}$, or sIgE$^{hi/lo}$ strata as the independent variable. *p*, by LRT. AA, allergic asthma; HDM, house dust mites; PARC, perennial allergic rhinoconjunctivitis

The proportion of individuals with sIgE^{hi} vs. sIgE^{lo} and neutrophil^{hi} vs. neutrophil^{lo} did not differ significantly by phenotype (Figure 5B); additionally, these variables did not significantly associate with differences in iSSS during or outside of the ACC (Figure 5C, Figures S8 and S9). The associations of these variables with individual components of iSSS are reported in Figures S6 and S8. Thus, in contrast to the CD8-CD4 balance clusters, the baseline eosinophil, neutrophil, and sIgE levels were not significantly associated with symptom severity during the ACC challenge (Figure 5C–E; Figures S6, S8 and S9).

While CD8-CD4^{hi} and eosinophil^{hi} were overrepresented in the maladaptive phenotype (Figure 5B), they had unequal contributions to disease severity in the ACC (Figure 5F). CD8-CD4 balance clusters explained nearly 18% of the variability in disease severity, whereas eosinophil, neutrophil, and sIgE levels explained a lesser amount and the associations were non-significant (Figure 5F). The ACQ-7 did not differ by CD8-CD4, eosinophil, or sIgE strata (Figure 4F, Figure S5). Collectively, these data (Figure 5) suggest that, while both CD8-CD4^{hi} and eosinophil^{hi} traits contribute to symptom responsiveness and the evoked phenotypes, the maladaptive phenotype is more closely aligned with the CD8-CD4^{hi} trait.

3.8 | Cohort 6. Immunophenotyping by evoked vs. cross-sectional phenotypes

Of the 86 immunologic traits evaluated (including CD4+ and CD8+ T-cell subsets, dendritic cells, monocytes, and B cells; Tables S8 and S9), only 24 subsets differed by evoked phenotypes at a false discovery rate <0.05 (Table S8). All of these traits related to CD4+ and CD8+ T-cell subsets. The maladaptive phenotype was characterized by an immune profile skewed toward activated effector memory, senescent, and terminally differentiated CD4+ and CD8+ T-cells (Figure S10). Figure 6 illustrates this skewing: levels of these lymphocyte subsets were (i) higher, intermediate, and lower in persons with the maladaptive, adaptive, and resilient phenotypes, respectively (top row); and (ii) higher in the CD8-CD4^{hi} and eosinophil^{hi} strata but not significantly different by sIgE strata (2nd-4th rows). The cross-sectional phenotypes misclassified the hierarchy of the immune traits associated with the evoked phenotypes (Figure 6, top- vs. bottom-most rows).

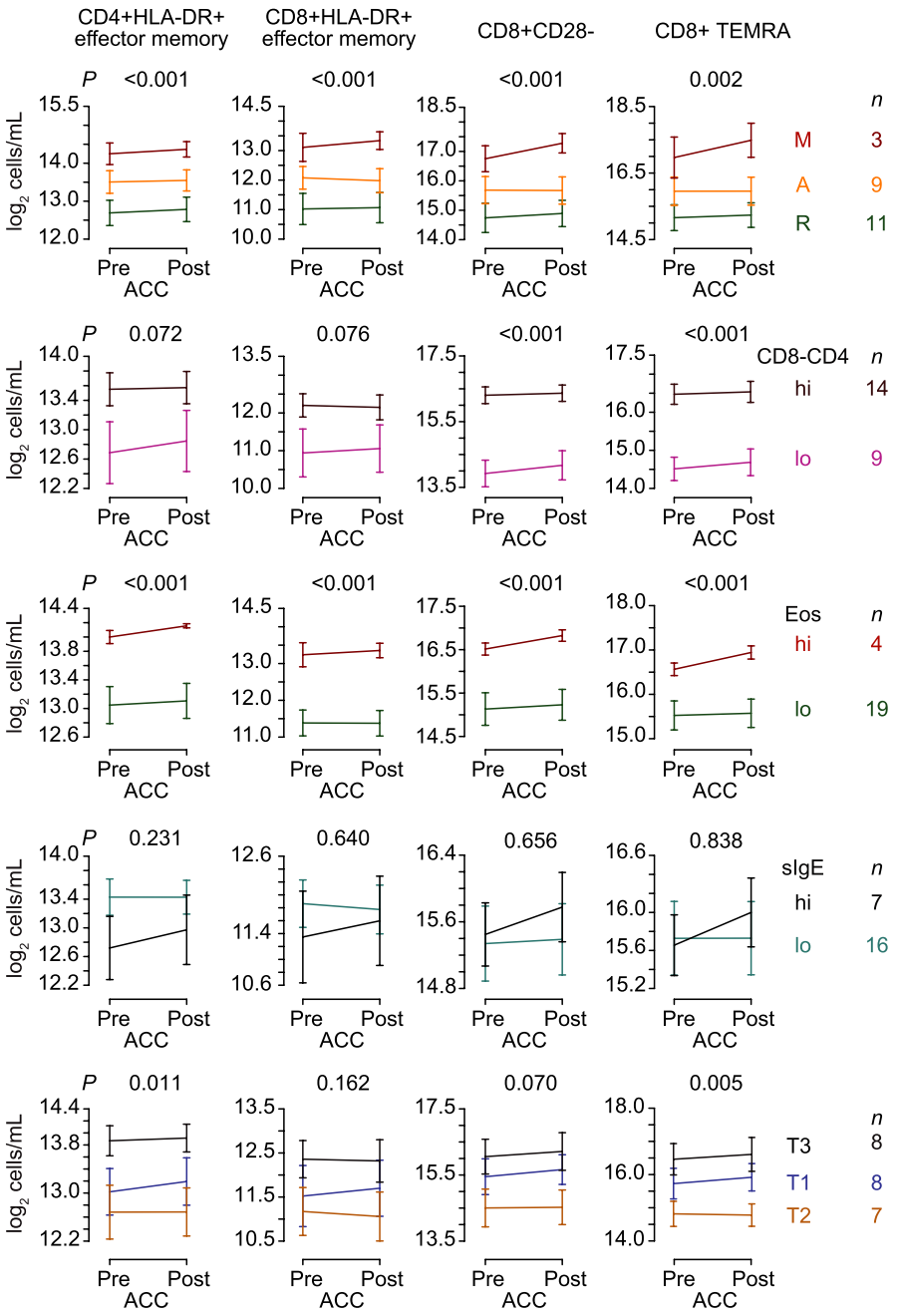


FIGURE 6 Peripheral blood T-cell traits by evoked phenotypes, peripheral blood trait strata, and cross-sectional phenotypes in HDM+PARC+AA+ participants challenged with house dust mites (HDMs) in an aeroallergen challenge chamber (ACC). Mean (SEM) of (*left to right)* CD4+HLA-DR+ effector memory, CD8+HLA-DR+ effector memory, CD8+CD28−, and CD8+ TEMRA T-cells before and after (pre vs. post) ACC exposure stratified by (*top to bottom)* evoked phenotypes (resilient [R], adaptive [A], maladaptive [M]), CD8-CD4 balance clusters, eosinophils (Eos), and HDM-specific IgE (sIgE) strata, and cross-sectional phenotypes (run-in rSSS tertiles T1 to T3 (T3 is the tertile with the upper-third values of the average-rSSS). *p*, by linear generalized estimating equation model with an exchangeable correlation structure and adjusted by time as a factorial variable with an analysis of variance (ANOVA). AA, allergic asthma; HDM, house dust mites; PARC, perennial allergic rhinoconjunctivitis

3.9 | Cohort 6. Nasal cell gene expression by evoked vs. cross-sectional phenotypes

The overrepresentation of CD8-CD4^{hi} and eosinophil^{hi} in the maladaptive phenotype (Figure 5B), as well as the associations of the evoked phenotypes with immune traits (Figure 6), prompted us to use a hypothesis-directed, non-agnostic approach to determine associations in gene expression profiles of nasal samples obtained from participants at three time points (t = 0, 2.5, and 5 h of ACC exposure; Figures 1D and 3A, left-bottom). This non-agnostic approach was based on the principle that our previous findings[8,10] and those of others[23–27] suggest that an imbalance between epithelial barrier function and inflammation influences AA pathogenesis (Figure 7A, model). We hypothesized that in response to ongoing epithelial injury, persons with AA mount an ineffectual attempt in the airway compartment to repair epithelial barrier; this triggers an allergic inflammation response mediated by several factors, including eosinophils and neutrophils (Figure 7A).

To test this hypothesis, we derived gene expression proxies to use in monitoring. The gene proxy used to monitor the epithelial injury/repair response was expression levels of filaggrin (*FLG*), a gene that plays a crucial role in epithelial barrier function.[28] The gene proxy for eosinophil-associated allergic inflammation[29–31] was periostin (*POSTN*), as it is intrinsically involved in many aspects of allergic inflammation, including eosinophil recruitment, airway remodeling, development of a Th2 phenotype, and increased expression of inflammatory mediators.[32] The gene proxy for neutrophil-associated inflammation was oncostatin M (*OSM*), as neutrophil-derived *OSM* influences various aspects of allergic inflammation.[33] The gene proxy

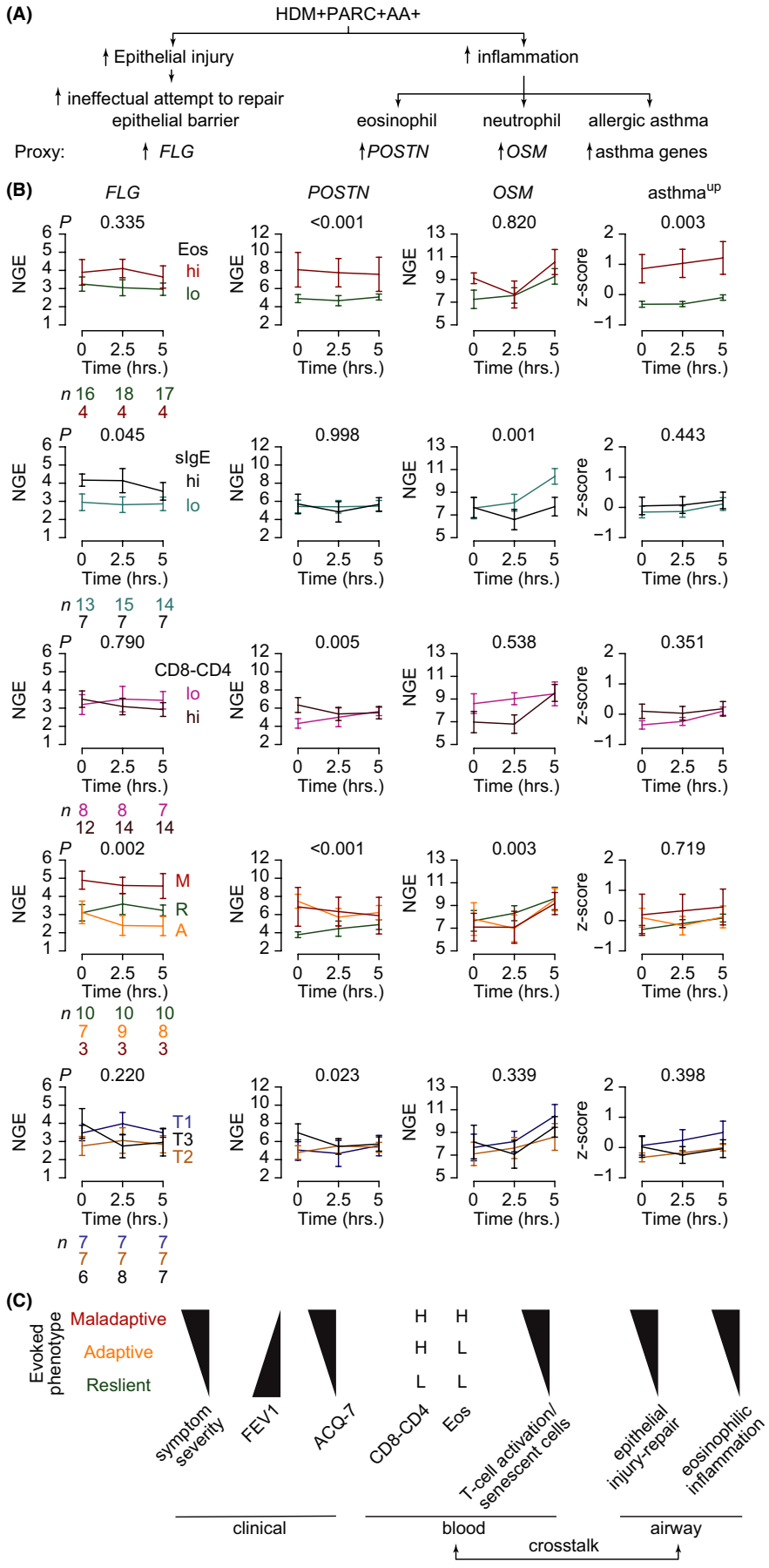


FIGURE 7 Nasal cell gene expression profiles in HDM+PARC+AA+ participants and summary mechanistic models. (A) Model of proxies for pathways influencing HDM-associated perennial allergic rhinoconjunctivitis with asthma (HDM+PARC+AA+) disease severity. (B) Gene expression of (*left to right*) *FLG*, *POSTN*, and *OSM* [$\log_2$-normalized gene expression (NGE)] and the asthma^{up} gene signature (*z*-scores) in nasal brushings at the indicated timepoints ($t = 0$, 2.5, and 5 h [hrs.]) in the ACC stratified by (*top to bottom*) eosinophil (Eos), HDM-specific IgE (sIgE), and CD8-CD4 balance cluster, evoked phenotypes (resilient [R], adaptive [A], maladaptive [M]), and cross-sectional phenotypes [run-in rSSS tertiles; T3 is the tertile with the upper-third values of the average-rSSS]. *p*, for NGE derived by a generalized estimating equation generalized linear model (GEE-GLM) based on the gamma distribution with an analysis of variance (ANOVA). *p*, for *z*-scores derived by a GEE-GLM based on the normal distribution and adjusted by time as a categorical variable with an exchangeable correlation structure with ANOVA. (C) Summary of traits (clinical, blood, and airway) associated with evoked phenotypes in HDM+PARC+AA+ persons. AA, allergic asthma; H, higher; HDM, house dust mites; L, lower; PARC, perennial allergic rhinoconjunctivitis

for allergic inflammation linked to asthma was an 18-gene signature (includes *POSTN* and *IL4*), designated as the asthma^{up} gene signature, that Poole et al. showed was upregulated in nasal cell transcriptomes of persons with asthma vs. controls.[34] We determined expression levels of these proxies in nasal cell transcriptomes according to whether they were indexed to (i) eosinophil^{hi} vs. eosinophil^{lo}, (ii) sIgE^{hi} vs. sIgE^{lo}, (iii) CD8^{hi}-CD4^{hi} vs. CD8^{lo}-CD4^{lo}, (iv) evoked phenotypes, or (v) cross-sectional phenotypes (Figure 7B).

The expression values of these genes across three timepoints are depicted (Figure 7B). Levels of *FLG*, *POSTN*, and *OSM*, but not the asthma^{up} gene signature, differed by evoked phenotypes; levels of *FLG* and *POSTN* were higher in the maladaptive vs. resilient phenotypes, whereas levels of *OSM* were lower. Congruent with the literature,[35,36] eosinophil^{hi} vs. eosinophil^{lo} associated with higher levels of the asthma^{up} signature as well as *POSTN*. sIgE^{hi} vs. sIgE^{lo} associated with higher levels of *FLG* and lower levels of *OSM* during the ACC exposure. CD8-CD4^{hi} vs. CD8-CD4^{lo} associated with lower levels of *OSM* at baseline. Congruent associations with the cross-sectional phenotypes were not observed (Figure 7B, bottom row).

4 | DISCUSSION

For rigor and establishing the generalizability of the concept of evoked phenotypes, we monitored symptom responses recorded in real-time by atopic individuals challenged with an array of aeroallergens in an ACC. Advantages of this experimental approach included: (i) uniform and controlled aeroallergen exposure in all study participants, (ii) no impact of inclement weather, (iii) mitigation of other confounding co-factors in the natural environment (eg, dust and temperature) that may influence symptom levels, (iv) ensured compliance and more accurate assessment of symptoms while being monitored and given specific symptom-recording instructions, and (v) mitigation of recall bias through use of instantaneously recorded symptom measures. Our findings provide a basis for delineating the host x environment interactions that may contribute to the wide inter-individual differences in disease severity among persons with ARC/AA, despite the ubiquity of environmental disease triggers.

A unifying interpretation of findings across six challenge cohorts is that exposure to ubiquitous aeroallergens elicits a relatively conserved range of evoked phenotypes. Reflecting the generalizability of the concept of evoked phenotypes, with the exception of cohort 3 (birch), the frequency of the maladaptive phenotype in the cohorts challenged with seasonal (cohorts 1, 2, 4, and 5) and perennial (cohort 6) aeroallergens was less than 33% of the study population: cohort 1 (Virginia live oak), 24%; cohort 2 (mountain cedar), 14%; cohort 4 (Timothy grass), 32%; cohort 5 (mountain cedar), 20%; and cohort 6 (HDM), 13%. The frequency of the maladaptive phenotype in the birch cohort was 58%. Thus, the maladaptive phenotype was more common after exposures to pollens that were not endemic to San Antonio, Texas (eg, birch and Timothy grass), the geographic region where the ACC challenge studies were performed. In contrast, the resilient and adaptive phenotypes appeared to be more common after exposure to endemic pollens (eg, mountain cedar and Virginia live oak). Hence, we suggest the following continuum: recurrent exposure to endemic aeroallergens in the environment may initially skew susceptible atopic persons toward the resilient and adaptive phenotypes corresponding to mild or moderate disease severity, respectively; a smaller subset develop the maladaptive phenotype. Underscoring this viewpoint, in a meta-analysis of three cohorts challenged with distinct seasonal aeroallergens only 6.5% (5 of 77) manifested the maladaptive phenotype, whereas 33.8% and 59.7% manifested the adaptive and resilient phenotypes, respectively. The proportions of the maladaptive, adaptive, and resilient phenotypes in the HDM+PARC+AA+ cohort were 13% (3 of 23), 39.1% (9 of 23), and 47.8% (11 of 23), respectively, and 30.4% (7 of 23), 30.4% (7 of 23), and 39.1% (9 of 23), respectively, in the HDM+PARC+AA− cohort that we described previously[8] (Figure 4G).

Thus, the findings presented herein and previously,[8,10] suggest that progression from less to more-severe ARC/AA may relate to the failure to preserve the resilient and adaptive phenotypes and this failure occurs in a small group of atopic individuals. In the seasonal aeroallergen cohorts, the maladaptive phenotype did not associate with polysensitization but did associate with higher levels of sIgE. Our mechanistic studies in HDM+PARC+ persons reported here, as well as previously,[8] suggest that this failure associated with a heightened inflammatory tone in the peripheral blood and airway compartments, as well as deficits in the epithelial injury/repair response. However, whether these changes presage and contribute to the development of the maladaptive phenotype vs. are a consequence of the maladaptive phenotype cannot be differentiated.

Among HDM+PARC+AA+ persons, symptom responsiveness in the ACC was more closely aligned to evoked phenotypes vs. constitutive cross-sectional phenotypes. The metrics commonly used to assess asthma severity and control displayed congruent hierarchal patterns among evoked phenotypes. For the maladaptive phenotype it was TASS^{higher}-TSS^{higher}-FEV1^{lower}-ACQ-7^{higher}, whereas for the adaptive vs. resilient phenotypes, the patterns were TASS^{intermediate}-TSS^{intermediate}-FEV1^{higher}-ACQ-7^{lower} vs. TASS^{lower}-TSS^{lower}-FEV1^{higher}-ACQ-7^{lower}, respectively (Figure 7C). However, since the cross-sectional phenotypes were a conflation of the evoked phenotypes, these hierarchal patterns of disease metrics were masked.

Among HDM+PARC+AA+ persons, the maladaptive phenotype associated with CD8-CD4^{hi}/eosinophil^{hi} attributes in the peripheral blood, whereas the adaptive and resilient phenotypes associated with CD8-CD4^{hi}/eosinophil^{lo} and CD8-CD4^{lo}/eosinophil^{lo}, respectively (Figure 7C). Similarly, the resilient, adaptive, and maladaptive phenotypes in HDM+PARC+AA+ persons associated with incrementally higher levels of activated, terminally differentiated, and senescent CD8+ T-cells (Figure 7C). In nasal cells, compared to the resilient phenotype the maladaptive phenotype associated with higher gene expression levels of *FLG*, a proxy for epithelial injury/repair, as well as *POSTN*, a marker for eosinophil-associated allergic inflammation. However, a consistent association of these mechanistic correlates with the cross-sectional phenotype was not observed.

In the HDM+PARC+AA+ cohort, we found that peripheral blood profiles signifying T-cell activation distinguished CD8-CD4^{hi} vs.

CD8-CD4^{lo} as well as eosinophil^{hi} vs. eosinophil^{lo} attributes; expression levels of the asthma^{up} gene signature in nasal cells was higher with eosinophil^{hi} vs. eosinophil^{lo}, whereas the *FLG* levels were higher with sIgE^{hi} vs. sIgE^{lo}. However, of these traits, only CD8-CD4^{hi} associated significantly with symptom severity, suggesting that the evoked phenotypes are likely the product of a complex coalescence of traits in the peripheral blood and airways as well as a crosstalk wherein peripheral blood inflammatory tone influences trait levels in the nasal compartment (Figure 7C). Thus, while most biological agents have been developed to mitigate Th2-biased inflammation, including eosinophil- and IgE-associated inflammation, in HDM+PARC+AA+ persons, we identified a significant contribution of peripheral blood CD8+ T-cell-associated inflammation. In our study, CD8+ T-cell levels, in conjunction with CD4+ T-cell levels (CD8-CD4^{hi}), explained a higher variability of disease severity than eosinophil, neutrophil, and HDM-specific IgE levels.

Hence, the juxtaposition of the findings of mechanistic studies performed previously in HDM+PARC+AA–[8] and currently in HDM+PARC+AA+ persons suggests that the failure to preserve the resilient and adaptive phenotype in HDM+PARC+ individuals may partly relate to increased CD8+ T-cell-associated inflammation. A role of CD8+ T-cells in AA pathogenesis has been noted in both murine and human studies.[37–49] In murine models, CD8+ T-cells have been identified as a source of type 2 inflammation (eg, IL-13, IL-4, and IL-5) and mediators of airway hyperresponsiveness.[39] Complementing these findings, depletion of CD8+ T-cells has been associated with beneficial effects, including attenuation of lung eosinophilia and airway hyperresponsiveness.[38,39] Corroborating our findings of increased effector memory CD8+ T-cells in the maladaptive phenotype, murine studies demonstrate that adoptive transfer of effector memory CD8+ T-cells can recapitulate allergic pathology.[40] Additional contributions of CD8+ T-cells in asthma are summarized (Note S3).

Our study has limitations. First, as cohorts 1–5 represent independent studies, the symptom severity metrics utilized for some studies varied (iTSS, iSSS, or iTNSS). However, these disease metrics and their components were highly correlated (Figure S11). Additionally, a meta-analysis of three cohorts supported that, while we lacked a uniform set of disease metrics across cohorts 1–5, the inferences were generalizable across the cohorts. Second, persons with severe HDM+PARC+AA+ were not evaluated in the ACC. However, this omission was due to the concern of evoking a severe asthma attack. In addition to safety concerns, results may have been confounded due to concomitant medication use (Note S4). However, both in the present study, as well as in our recent report,[8] many of the clinical and mechanistic correlates associated with the maladaptive phenotype suggest that this phenotype may serve as a proxy for severe AA. This viewpoint is consistent with the inferences of a recent study of persons with asthma phenotyped following an experimental challenge with rhinovirus.[50] Third, we were unable to sequentially challenge the same group of individuals with different seasonal aeroallergens across multiple years. Nonetheless, the generalizability of the evoked phenotypes observed in multiple cohorts suggests that our findings are not spurious. Fourth, we did not evaluate persons who initially had the resilient and adaptive phenotype and then prospectively over time developed the maladaptive phenotype. Thus, a cause (failure to preserve resilient/adaptive phenotype) and effect (development of maladaptive phenotype) relationship cannot be established. Finally, the proportion of individuals in the HDM+PARC+AA+ cohort ($n = 23$) who elicited the maladaptive phenotype was small (13%; $n = 3$). However, this was to be anticipated, as per our model, most individuals preserve mechanisms to manifest the resilient and adaptive phenotype. Corroborating this viewpoint, after challenge with HDM, only 30.4% ($n = 7$) of a cohort of HDM+PARC+AA− individuals ($n = 23$) manifested the maladaptive phenotype (Figure 4G).[8] Additionally, mitigating the concern of sample size, the phenotyping in the ACC provides a more accurate and precise assessment of disease severity and associated mechanisms. Indeed, artificial inflation of the more-severe group sample size by use of the run-in tertiles to categorize disease severity (cross-sectional phenotypes) would have masked both the true level of disease severity evoked after challenge as well as mechanistic correlates. Similar masking was observed in our previous challenge studies in HDM+PARC+AA− individuals.[8]

In summary, results of the challenge studies presented here support the use of the ACC for precision phenotyping to determine mechanisms that may track persons who preserve the resilient or adaptive phenotype versus those who may have shifted to the maladaptive phenotype. In HDM-sensitive persons, the trifecta of increased CD8+ T-cell-associated inflammation, deficits in the epithelial injury/repair response, and other maladaptive responses may contribute to the development of severe AA, tracked by the maladaptive phenotype. However, larger ACC exposure studies are warranted to further explore how the coalescence of multiple traits and intercompartment crosstalk (Figure 7C) contribute to the development of the maladaptive phenotype.

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CONFLICT OF INTEREST

RLJ is the owner of the Biogenics Research Chamber. The rest of the authors declare that they have no relevant conflicts of interest.

AUTHOR CONTRIBUTIONS

RLJ, SKA, RMR, and AMS designed the research studies; AMS, NH, FJ, APB, JAM, LP, AC, CW, LW, and MSM performed the experiments or bioinformatic/statistical analysis; AMS, NH, FJ, JAM, APB, MSM, and SKA analyzed and interpreted the data; RLJ, RMR, CGR, DAR, and CPA performed the chamber runs. SKA, RLJ, AMS, RMR, NH, FJ, APB, JAM, LP, AC, CW, LW, MIR, DJM, JAP, RAC, GCL, AGM, MSM, and JFO contributed to the ideas tested in the study. SKA wrote the manuscript with contributions from RLJ, AMS, RMR. SKA, RLJ, and JFO obtained the primary funding for the studies.

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