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

Short title: Maladaptive responses to aeroallergens track severe allergic airway disease

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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).

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 nonendemic 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 (i.e., ACC), most atopic persons manifest the resilient or adaptive phenotype. Thus, ARC/AA disease progression may reflect the failure to preserve the resilient/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, epithelium, rhinoconjunctivitis, phenotypes, T-cells

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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
slgE, HDM-specific IgE
SSS, summated symptom scores
TASS, total asthma symptom scores
TNSS, total nasal symptom scores
TOSS, total ocular symptom scores

TSS, total symptom scores

Th2, T helper-2

VLO, Virginia live oak

INTRODUCTION

The two-compartment (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 (e.g., Mountain cedar) and perennial (e.g., 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**). 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 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 disease severity (mild vs. moderate vs. severe) and/or biomarker levels (e.g., T helper-2 [Th2]-high asthma) recorded in the resting state (i.e., 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 the natural environment as well as other environmental factors (e.g., dust, temperature) that may influence symptom severity⁵. 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 study⁸, 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, respectively⁸. Those studies also supported the idea that the constitutive cross-sectional phenotypes group persons with different evoked phenotypes⁸ (e.g., **Figure 1B**). This grouping masked the true mechanistic correlates of disease severity in HDM+PARC+AA– persons⁸.

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 to 8 hours) and frequency (1 to 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 study⁸ 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

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monitoring, we restricted the (i) number of HDM+PARC+AA+ persons who were simultaneously exposed to HDMs, (ii) challenge to a single 5-hour 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 (e.g., cold air, viral infection) triggers of ARC/AA were not high.

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-S2** and the supplementary methods.

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 asthma⁸].

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 previously⁸.

ACC operations

The ACC facility, operations, as well as methods for aeroallergen dispersal and monitoring are described (**supplementary note 1**). The exposure regimens are depicted (**Figure 1C,D**)

and the challenge protocols were synonymous amongst each other and with our previous studies⁸⁻¹⁴. The only exception is for cohort 3, as this study was performed during the COVID-19 pandemic; the ACC was modified to ensure safety (**supplementary note 1**). 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 generated⁵ (**Table S1**).

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 practices¹⁷⁻¹⁹, instantaneous and reflective symptom scores recorded by the participant are indicated by the prefixes i and r, respectively. The prefix i before a metric (e.g., iSSS) indicates that the score was recorded instantaneously at defined intervals in the ACC and represents the symptoms experienced over the past 10-15 minutes. In contrast, the prefix r (e.g., 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-hour 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.

Evoked phenotypes in cohorts 1-5: seasonal aeroallergens

Symptom metrics recorded at 30-minute intervals during the ACC exposures were used for clustering analyses (unsupervised hierarchical clustering with Ward's D linkage and Manhattan distances; supplementary methods). 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-hour aeroallergen exposure (day 1) and subsequent exposures (days 2, 3, and 4) which were longer (8 hours/day) and occurred on consecutive days (**Figure 1C**). In contrast, aeroallergen exposures in cohorts 1-4 were for 3 hours/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 (supplementary methods).

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

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 $\geq 12\%$ 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 (supplementary methods): 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-hour exposure to HDM powder (**Figure 1D**; **Figure S1B**). Participants recorded symptom severity metrics and FEV1 during the run-in phase (AM and PM), ACC exposure (at 30-minute intervals), and for four 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.

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

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) (visit 2; **Figure 1D**), using methods described previously^{8,10}. Eighty-seven 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/ μ L, neutrophil^{hi}: 1×10^6 cells/mL, and sIgE^{hi}: 0.35 kU/L. The rationale for the cutoffs is outlined (supplementary methods).

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-minute 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 phenotype.

Nasal cell gene expression profiling. Nasal brushings were obtained before, in the middle, and after the ACC challenge ($t=0$, 2.5 and 5 hours, 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} (supplementary methods). Expression of genes relevant to AA was monitored via the asthma^{up} 18-gene signature (supplementary methods).

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 paired t-test, Fisher's exact test, z-transformation, linear regression, likelihood ratio test (LRT), and analysis of variance (ANOVA). For analysis of repeated measurements from the sample 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 (e.g., iTNSS-AUC, iTSS-AUC, iSSS-AUC, 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.

RESULTS

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.

Evoked phenotypes in response to seasonal aeroallergens: cohorts 1-5

The symptom responses evoked during two shorter (3 hours/each; cohorts 1-4) as well as prolonged and repeated (cohort 5) exposures to seasonal aeroallergens was analyzed (**Figure 1C; Figure 2**). At baseline in the ACC (i.e., 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-hour exposure to mountain cedar (**Figure 2E**, top left). In the subsequent 8-hour 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.

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, i.e., 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 grouped within the resilient phenotype. Similar post hoc analysis in cohorts 1 and 2 also did not identify an adaptive group conflated within the resilient phenotype (**Figure 2A,B**, right). Thus, results of these post hoc analyses highlight the fidelity of our clustering approach for assignment of phenotypes and suggest that the 2-cluster response observed in participants challenged with Timothy grass was unlikely to be spurious.

Second, nearly 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 (San Antonio, Texas). Exposure to a non-endemic vs. endemic (e.g., 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.

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-1 and resilient-2 groups; **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 33.7%, 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; **supplementary note 2**). 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-1 (A1) and the maladaptive phenotype (**Figure 2H**, bottom). Notably, these two phenotypes associated with higher symptom responsiveness during the first ACC challenge (**Figure 2G**).

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 nonendemic (Timothy grass) pollens.

Cohort 6. HDM-PARC+AA+ phenotypes

Twenty-three HDM-PARC+AA+ participants completed the 5-hour exposure to HDMs (**Figure S1A-B**); one participant was removed from the ACC based on protocol specifications (a decrease in FEV1 of $\geq 12\%$ experienced on two occasions during the ACC that was not reversible with nebulized bronchodilators; supplementary methods). While the mean iSSS increased in response to HDM exposure (**Figure 3A**; left; $P < 0.001$), 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 demographic 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 low²¹; correspondingly, aside from a weak correlation with iTASS ($r=-0.31$, $P=0.157$), 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) 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) 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.

Cohort 6. Confounding with constitutive cross-sectional phenotyping

To examine whether symptom severity recorded in the natural setting before ACC challenges (constitutive cross-sectional phenotype) 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**; summarized in **Figure 4B,4E**).

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, while an average run-in rSSS ≥ 19.31 or baseline ($t=0$) FEV1 ≤ 2.84 would have distinguished persons with the maladaptive vs. the resilient and adaptive phenotypes (**Figure 3B,4E**), cutoffs that distinguish the resilient vs. adaptive phenotypes were not evident without an overlap in these metrics between these two phenotypes. 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 previously⁸ (**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 previously⁸, use of the run-in tertiles would have masked this stereotypical evoked response.

Cohort 6. Conventional laboratory measures and symptom severity

Eosinophils, neutrophils, and IgE have established roles in ARC/AA pathogenesis²². 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 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 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**).

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-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 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-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 clusters explained nearly 18% of the variability in disease severity, whereas eosinophil-, neutrophil-, and sIgE levels explained a lesser amount and the associations were nonsignificant (**Figure 5F**). The ACQ-7 did not differ by CD8-CD4, eosinophil, or sIgE strata (**Figure 5E**, **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 CD8-CD4^{hi} trait.

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

Of the 87 immunologic traits evaluated (including CD4+ and CD8+ T-cell subsets, dendritic cells, monocytes, and B cells; **Tables S8-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).

Cohort 6. Nasal cell gene expression by evoked vs. cross-sectional phenotype

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 hours of ACC exposure; **Figure 1D**, **Figure 3A**, left-bottom). This non-agnostic approach was based on the principle that our previous findings^{8,10} and those of others²³⁻²⁷ 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 function²⁸. The gene proxy for eosinophil-associated allergic inflammation²⁹⁻³¹ 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 mediators³². The gene proxy for neutrophil-associated inflammation was oncostatin M (*OSM*), as neutrophil-derived *OSM* influences various aspects of allergic inflammation³³. The gene proxy 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. controls³⁴. 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) slgE^{hi} vs. slgE^{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*. slgE^{hi} vs. slgE^{lo} associated with higher levels of *FLG* and lower levels of *OSM* during the ACC exposure. CD8^{hi}-CD4^{hi} vs. CD8^{lo}-CD4^{lo} associated with higher levels of *OSM* at baseline. Congruent associations with the cross-sectional phenotypes were not observed (**Figure 7B**, bottom row).

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 (e.g., dust, 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) instantaneously recorded symptom measures mitigate recall bias. 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, the frequency of the maladaptive phenotype in the cohorts challenged with seasonal (cohorts 1-5) 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 3 (birch), 13%; cohort 4 (Timothy grass), 32%; cohort 5 (mountain cedar), 20%; and cohort 6 (HDM), 13%. Thus, the maladaptive phenotype was more common after exposures to pollens that were not endemic to San Antonio, Texas (e.g., 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 (e.g., mountain cedar, 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), whereas 33.8% and 59.7% manifested the adaptive and maladaptive phenotypes, respectively. The corresponding proportions in the HDM+PARC+AA+ cohort were 13%, 39.1%, and 47.8%, respectively. Similar proportions were observed in the HDM-PARC+AA- cohort previously reported on⁸ (**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 previously⁸, suggest that this failure associates 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, 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 suggest that the failure to preserve the resilient and adaptive phenotype in HDM-PARC individuals may partly relate to increased CD8+ T-cell activation. A role of CD8+ T-cells in AA pathogenesis has been noted in both murine and human studies³⁷⁻⁴⁹. In murine models, CD8+ T-cells have been identified as a source of type 2 inflammation (e.g., IL-13, IL-4, IL-5) and mediators of airway hyperresponsiveness³⁹. 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 pathology⁴⁰. Additional contributions of CD8+ T-cells in asthma are summarized (**supplementary note 3**).

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 (**supplementary note 4**). However, both in the present study, as well as in our recent report⁸, 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 rhinovirus⁵⁰. 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 suggest 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 22% ($n=5$) 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 of mechanistic was observed in our previous challenge studies in HDM+PARC+AA– individuals⁸.

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**) contributes to the development of the maladaptive phenotype.

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FIGURE LEGENDS

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 1 second; HDM, house dust mite; hr, hour; M, maladaptive; MC, mountain cedar; R, resilient; sIgE, house dust mite-specific IgE; SSS, summated symptom score; t, timepoint; TNSS, total nasal symptom score; TSS, total symptom score; VLO, Virginia live oak.

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-minute 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-minute 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-minute intervals during exposure 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}: 0.35kU/L (supplementary methods). **(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*<0.05, **, *P*<0.01, ***, *P*<0.001, ns, nonsignificant.

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. (A) Left: Mean (SEM) instantaneous summated symptom scores (iSSS) at 30-minute intervals in HDM-PARC+AA+ participants during a 5-hour 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 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 $\geq 12\%$ (supplementary methods). *P*, by Fisher's exact test. AA, allergic asthma; FEV1, Forced expiratory volume in the first second; L, liters.; PARC, perennial allergic rhinoconjunctivitis.

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. (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 highest tertile]. 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-AUC in the ACC by evoked phenotypes and cross-sectional phenotypes. *P*, by GEE model with an exchangeable correlation structure. **(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 cut-offs in supplementary methods. *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.

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 (slgE) strata. Strata cut-offs in supplementary methods. *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 slgE^{hi/lo} strata as the independent variable. *P*, by LRT. AA, allergic asthma; HDM, house dust mites; PARC, perennial allergic rhinoconjunctivitis.

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 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 in stratified by (*top to bottom*) evoked phenotypes (resilient [R], adaptive [A], maladaptive [M]), CD8-CD4 balance clusters, eosinophils (Eos), and HDM-specific IgE (slgE) strata, and cross-sectional phenotypes (run-in rSSS tertiles T1 to T3 (T3 is highest tertile)). *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.

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 hours [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 [Tert]; T3 highest tertile)]. *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.

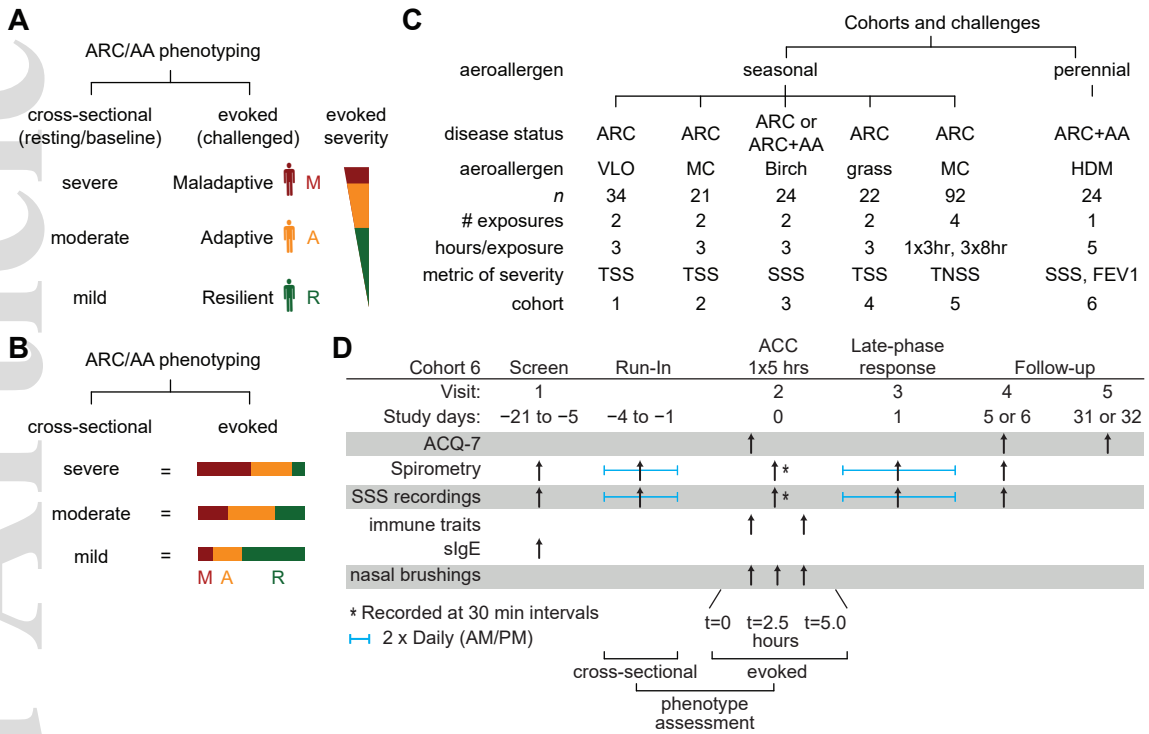
FIGURE 1.

FIGURE 2.

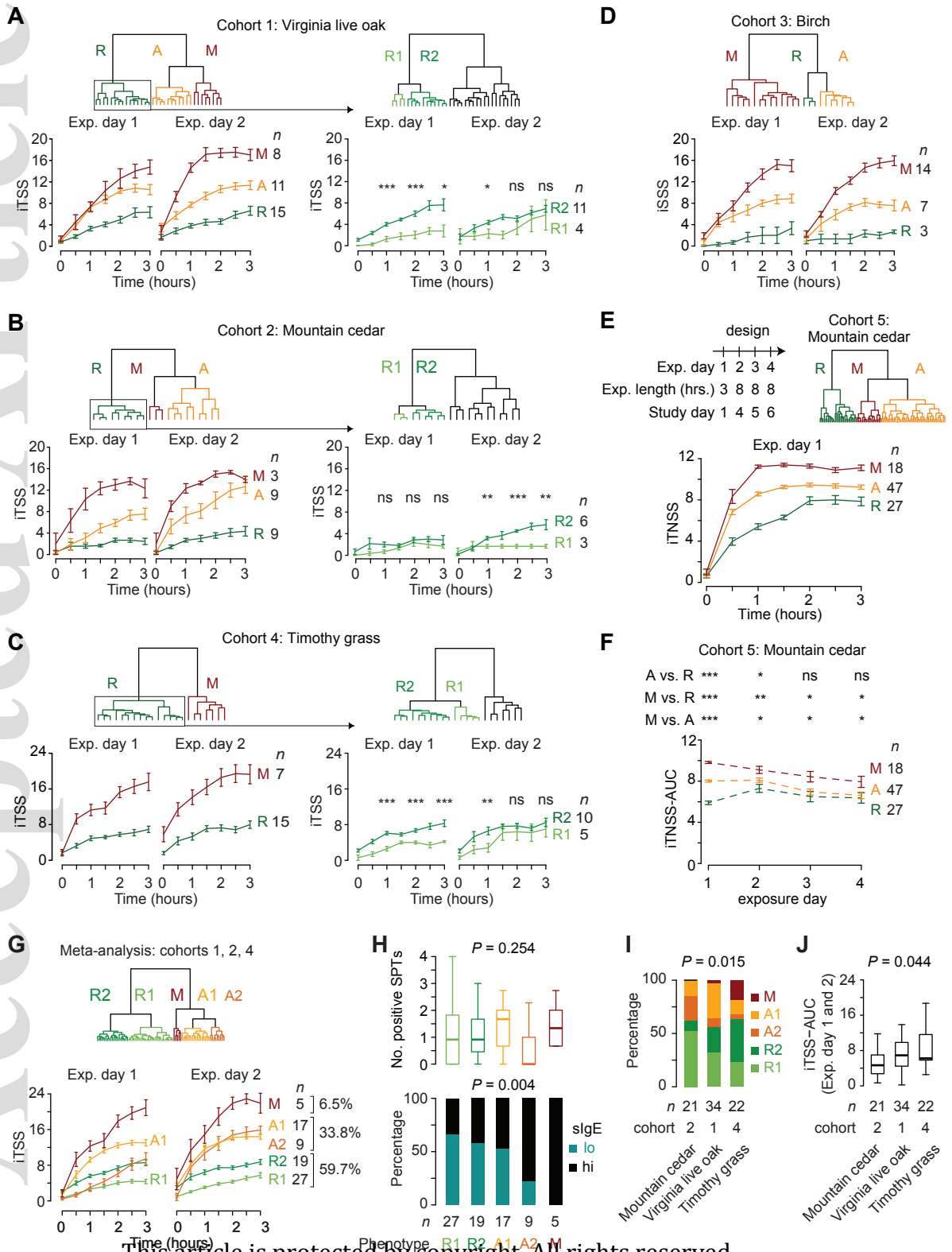


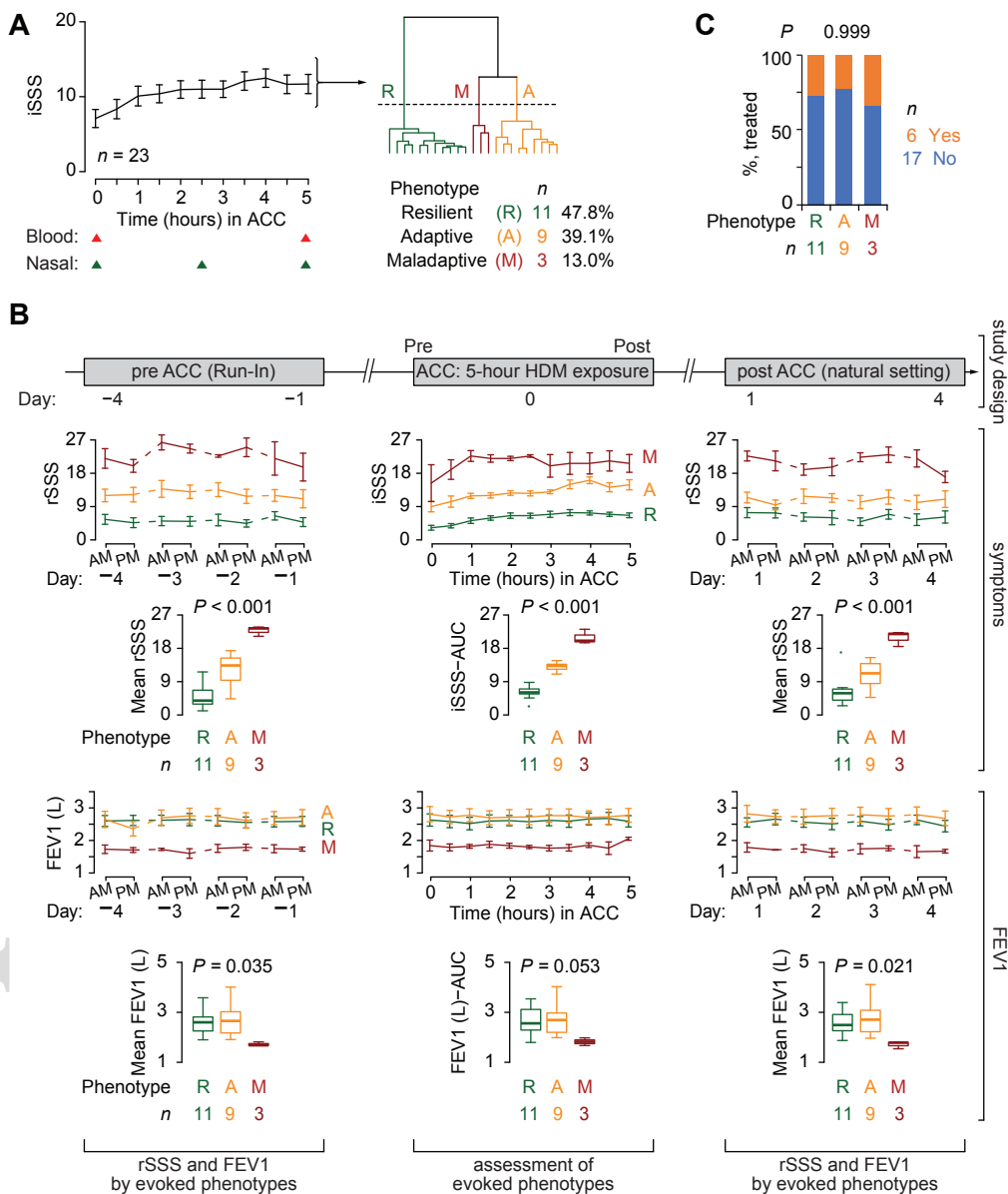
FIGURE 3.

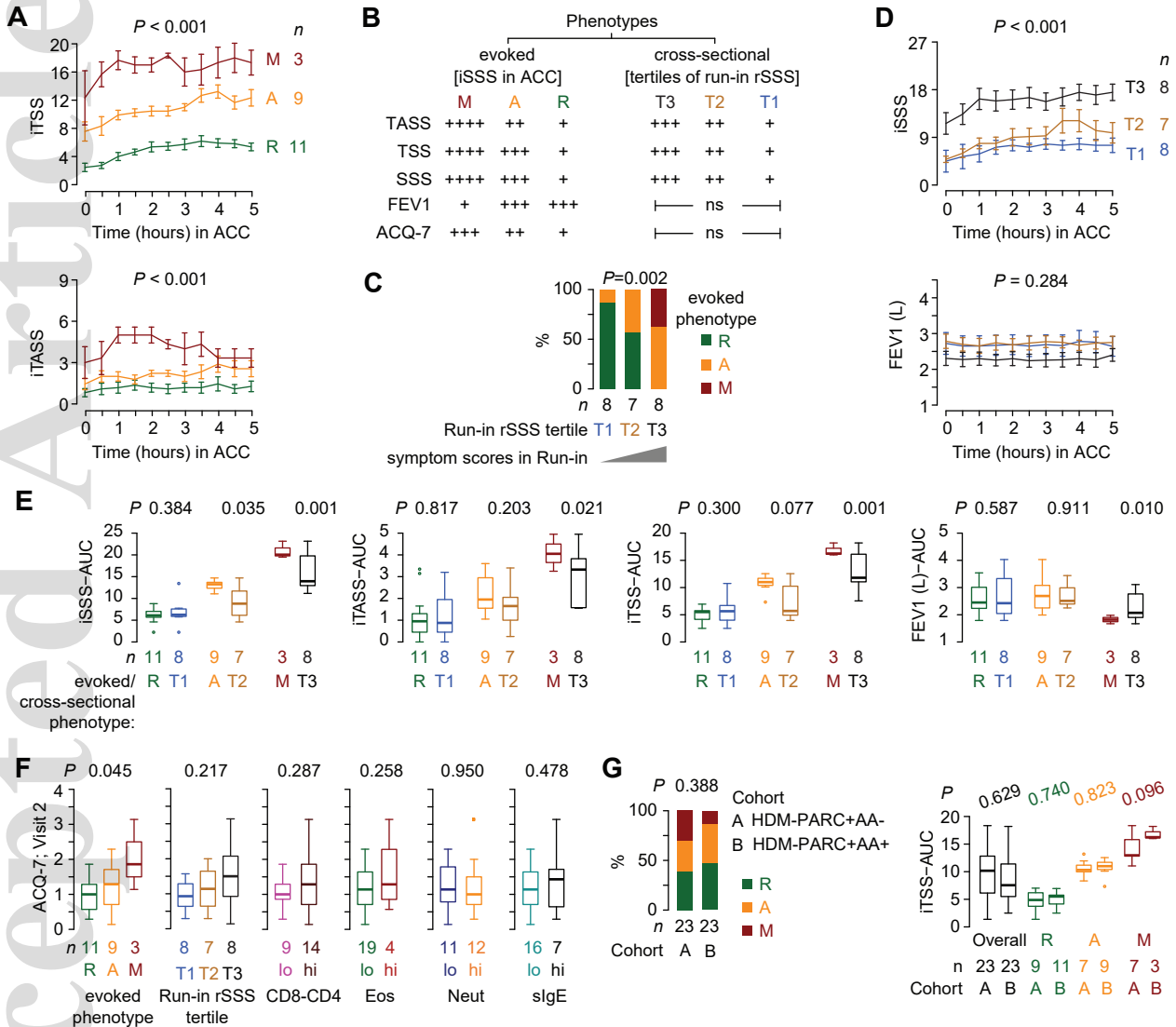
FIGURE 4.

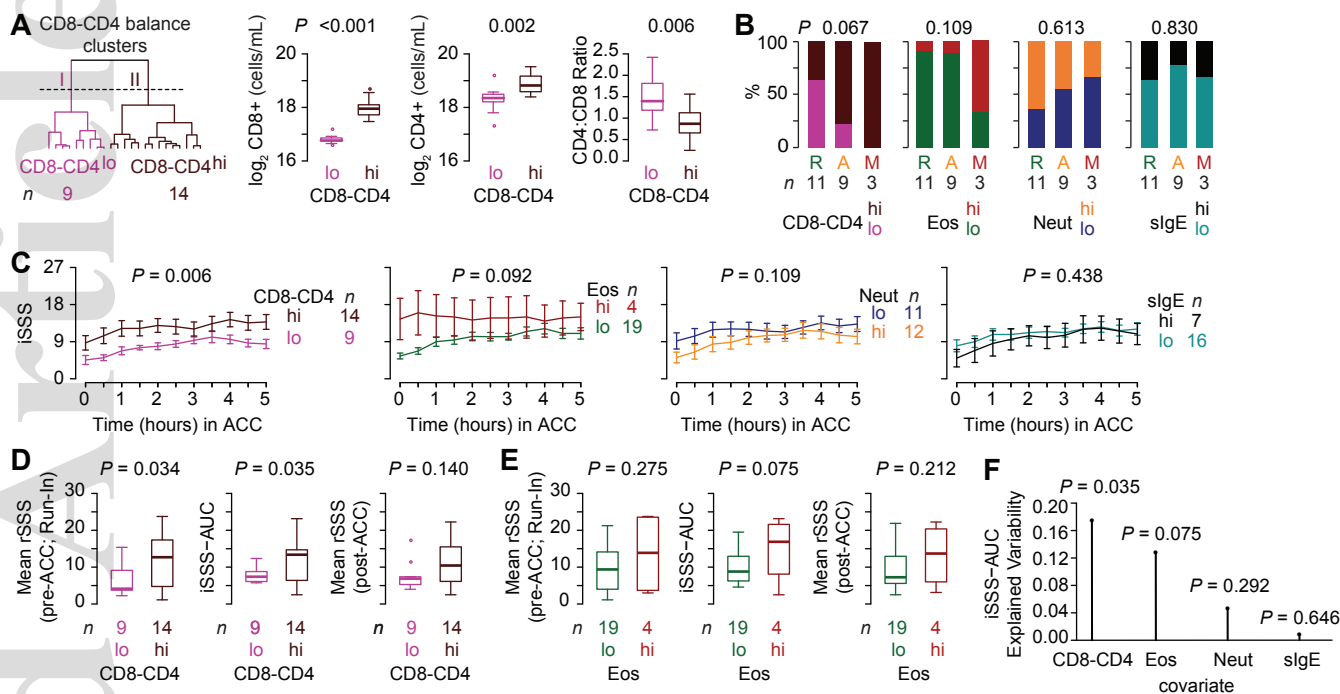
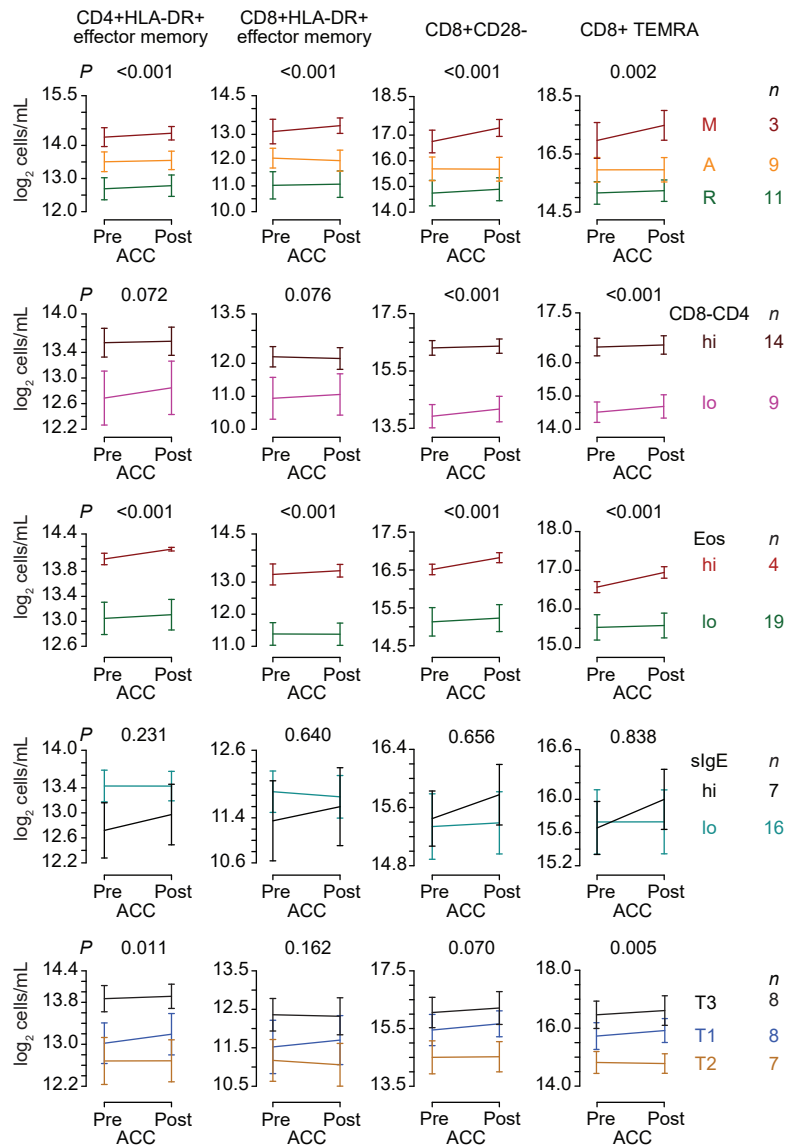
FIGURE 5.

FIGURE 6.



A

HDM-PARC+AA+

↑ Epithelial injury
↓
↑ ineffectual attempt to repair epithelial barrier
Proxy: ↑ *FLG*

↑ inflammation
↓
eosinophil ↑ *POSTN*
neutrophil ↑ *OSM*
allergic asthma ↑ asthma genes

B

FLG *POSTN* *OSM* asthma^{UP}

NGE *P* 0.335 *Eos* *hi* *lo* 0.003

Time (hrs.)

n 16 18 17
4 4 4

NGE *P* 0.045 *slgE* *hi* *lo* 0.443

Time (hrs.)

n 13 15 14
7 7 7

NGE *P* 0.790 *CD8-CD4* *lo* *hi* 0.351

Time (hrs.)

n 8 8 7
12 14 14

NGE *P* 0.002 *M* *R* *A* 0.719

Time (hrs.)

n 10 10 10
7 9 8
3 3 3

NGE *P* 0.220 *T3* *T2* 0.398

Time (hrs.)

n 7 7 7
6 8 7

C

Evoked phenotype

Maladaptive
Adaptive
Resilient

clinical blood cross-talk airway

symptom severity FEV1 ACQ-7 CD8-CD4 Eos T-cell activation/senescent cells epithelial injury-repair eosinophilic inflammation

Online supplementary material for

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

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Supplementary Methods

A. Cohorts 1-5: study design and participants

A.1. Overview.

Cohorts 1-5 were studied here solely to test whether the resilient, adaptive, and maladaptive evoked phenotypes extend to persons challenged with an array of seasonal aeroallergens (**Table S1**),

A.2. Cohort 1.

Cohort 1 inclusion/exclusion criteria, characteristics, and the related study details and design have been detailed previously¹. Briefly, cohort 1 comprised 34 persons with positive skin prick test (SPT) reactivity (≥ 5 mm compared to saline control) to Virginia live oak (VLO), a history of ARC, and without physician-diagnosed asthma; overall cohort and by evoked phenotype demographics are outlined in **Table S10**. The study design consisted of two consecutive daily exposures to VLO each 3 hours (**Figure 1C**). Data available to for analysis was the instantaneous TSS at 30-minute intervals from time=0 hours to time=3 hours using a Likert-scale of 0-3 to score each component of TSS (**Table S4**).

A.3. Cohort 2.

Cohort 2 inclusion/exclusion criteria, characteristics, and the related study details and design have been detailed previously¹. Briefly, cohort 2 comprised 21 persons with positive SPT reactivity (≥ 3 mm compared to saline control) to Mountain Cedar (MC), a history of ARC, and without physician-diagnosed asthma; overall cohort and by evoked

phenotype demographics are outlined in **Table S10**. The study design consisted of two consecutive daily exposures to MC each 3 hours (**Figure 1C**). Data available for analysis was the instantaneous TSS at 30-minute intervals from time=0 hours to time=3 hours using a Likert-scale of 0-3 to score each component of TSS (**Table S4**).

A.4. Cohort 3.

Cohort 3 inclusion/exclusion criteria and characteristics have not been previously described and thus are outlined below and in **Table S10**. Participants were recruited for a single-site study conducted in the ACC to evaluate symptom responses to birch pollen exposure. The study aimed to recruit 25 generally healthy male and female adult subjects with a history of seasonal ARC, with or without asthma, to VLO pollen and a positive skin prick test to both VLO and birch pollen extract. To assess natural exposure to birch pollen all subjects responded to a questionnaire concerning places of residency throughout their lives. Thirty VLO pollen allergic and birch pollen sensitive subjects, males and females, 18-60 years of age were pre-screened; 25 persons qualified for the study.

Eight of the twenty-four subjects had documented asthma (**Table S10**; Cohort 3) by history and FEV1 reversibility $\geq 12\%$ in response to pre- and post-bronchodilator spirometry. One subject, with known hypertension, was withdrawn from the study before entry into the ACC because of elevated blood pressure. There were no other adverse events. Serum-specific IgE (ssIgE) for VLO and birch pollen was collected from all participants. Sixteen of 24 subjects had a measurable ssIgE. Twelve subjects had a

sslgE to VLO pollen of Class II or greater; 10 of these subjects had a sslgE to birch pollen of Class II or greater. Fifteen subjects had no previous natural exposure to birch pollen.

The study design consisted of two consecutive daily exposures to Birch pollen each 3 hours (**Figure 1C**). The first chamber exposure was initiated with birch pollen at an average concentration of $3,500 \pm 750$ grains/m³. Subjects exited the chamber and returned 21 hours later for the second birch pollen exposure which was conducted using the same protocol as the first exposure. Twenty-four subjects completed the study; none of the subjects experienced bronchospasm within either of the chamber exposures. Data available for analysis was instantaneous total symptom scores (TSS; **Table S4**) as well as asthma symptoms of wheezing, cough, and dyspnea measured by the total asthma symptom score (TASS; **Table S4**) at baseline and 30-minute intervals throughout each of the 3-hours exposures on a scale of 0-3 using a Likert scoring chart. Thus, persons of cohort 3 were evaluated based on the summated symptom score (SSS) metric (**Table S4**).

A.5. Cohort 4.

Cohort 4 inclusion/exclusion criteria, characteristics, and the related study details and design have been detailed previously². Briefly, cohort 4 comprised 22 persons with positive SPT reactivity (≥ 5 mm compared to saline control) to Timothy grass, a history of ARC, and without physician-diagnosed asthma; overall and evoked phenotype demographics are outlined in **Table S10**. The study design consisted of two consecutive

daily exposures to grass each 3 hours (**Figure 1C**). Data available for analysis was the instantaneous TSS at 30-minute intervals from time=0 hours to time=3 hours using a Likert-scale of 0-3 to score each component of TSS (**Table S4**).

A.6. Cohort 5.

Cohort 5 inclusion/exclusion criteria and characteristics are outlined (**Table S10**). Cohort 5 was a part of a clinical study that is not yet published, and thus, limited phenotyping and symptom data are available for analysis. Cohort 5 comprised 92 persons with positive SPT reactivity (≥ 5 mm compared to saline control) to Mountain Cedar (MC), a history of ARC, and without physician-diagnosed asthma. The study design consisted of 1 priming exposure (3 hours) and three days later, 3 consecutive daily exposures (9 hours) to MC of which 8 hours were used for evaluation prior to initiation of an intervention unrelated to this study (**Figure 1C**). Data available for analysis was instantaneous Total Nasal Symptom Scores (TNSS; **Table S4**) at 30-minute intervals from time=0 hours to time=3 hours using a Likert-scale of 0-3 to score each component of TNSS.

B. Cohorts 1-5: evoked phenotypes and disease severity metrics

B.1. Hierarchical clustering (Cohorts 1-5)

Unsupervised hierarchical clustering with Ward's D linkage and Manhattan distances were used to derive evoked phenotypes. To derive evoked phenotypes: 14 (exposure days 1 and 2) iTSS (cohorts 1, 2, 4), 14 (exposure days 1 and 2) iSSS (cohort 3), and 7 (exposure day 1) iTNSS (cohort 5) participant-recorded measures recorded at 30-

minute intervals during the ACC exposures (as specified) were used for clustering (Table S1). Cohort 5 was an exception to the 14-measure clustering method since the cohort 5 study design was used to evaluate identified phenotypes across prolonged and repeated exposures (exposure day 1 phenotypes evaluated across exposure days 2, 3, and 4). A meta-analysis was performed in cohorts 1, 2, and 4; clustering was performed as described above using the 14 (exposure days 1 and 2) iTSS participant-recorded measures recorded at 30-minute intervals during the ACC exposures.

B.2. iTNSS-AUC calculation (Cohort 5)

To represent overall disease severity by a single metric, an area-under-the-curve (AUC) statistic was used. For calculating iTNSS-AUC, the iTNSS vs. time (hours) line was plotted for each participant, and the AUC was calculated for each participant using the trapezoid method and then divided by 3 (hours) to rescale the value into the same range as the iTNSS.

C. Cohort 6: HDM-PARC+AA+

C.1 Study participants and design

Characteristics of the HDM-PARC+AA+ participants and detailed inclusion/exclusion criteria are outlined (Tables S3, S6, S7). Table S11 details a list of medications that were restricted during the study except as prescribed by the principal investigator as a rescue medication. Due to uncertainty of the level of the asthmatic response that we might observe in the ACC, we implemented conservative safety protocols and recruited

persons with intermittent mild to moderate asthma, by GINA criteria³. Forty-four persons were screened of which 27 passed inclusion/exclusion criteria; of these 27 subjects, 17, 6 and 4 classified as having mild-intermittent, mild-persistent, and moderate asthma, respectively. Before the ACC visit (visit 2), three subjects were disqualified because of a positive pregnancy test, sinus infection, and principal investigator discretion (**Figure S1A**). Thus, 24 persons with (i) skin prick test (SPT) wheal reactivity to *Dermatophagoides pteronyssinus* (HDMs), (ii) history of perennial HDM allergy, (iii) physician-diagnosed asthma, and (iv) FEV1 reversibility $\geq 12\%$ at screening who met the inclusion/exclusion criteria participated in the study.

The study consisted of five study visits. Visit 1 (study days -21 to -5): screening and recruitment; Visit 2 (study day 0) was one 5-hour exposure to HDMs in the ACC; Visit 3 (study day 1) was late-phase response visit; and Visits 4 and 5 (study days 5 and 31, respectively) follow-up visits. The ACC used in this study had a maximum capacity of twelve persons, thus, participants were split into two groups (**Figure S1B**). Each group followed the same study design regimen and the studies were conducted one week apart. Participants recorded symptom scores (**Table S4**) instantaneously and spirometry in the ACC (at 30-minute intervals) as well as reflective symptom scores and spirometry in subject symptom diaries (twice daily AM/PM) during the 4-day run-in period prior to Visit 2 (study days -4 to -1) and in the natural setting after the ACC Visit 2 (during a four day contiguous stretch after the ACC; AM/PM) (**Figure 1D**). Asthma control questionnaire-7 assessments (ACQ-7) were completed at visit 2 (prior to HDM exposure), and visits 4 and 5. Peripheral blood was drawn at visit 1 and visit 2 (t=0 and

5 hours relative to initiation of HDM dispersal in the ACC). Nasal brushings were obtained at visit 2 (t=0, 2.5, and 5 hours relative to initiation of HDM dispersal in the ACC). The IntegReview Institutional Review Board (Austin, TX) approved the study (the Biogenics Research Chamber HDM/Asthma study 002) and all participants provided informed consent.

C.1.a ACC study design for HDM-PARC+AA+ participants

Methods for delivery, collection, and quantification of HDMs in the ACC were akin to our previous studies^{4,5}. Three collection stations were configured in a triangle in the ACC room and HDM samples were collected at 10, 60, 120 and 180, 240, and 300 minutes after initiating dispersal of the HDM milled powder into the ACC; Der p 1 levels were measured (**Table S12**). The HDM exposure in this study comprised one 5-hour exposure to finely-ground HDM body powder (*Dermatophagoides pteronyssinus*; Greer Laboratories). Participants recorded FEV1, and instantaneous total asthma, nasal, and ocular symptoms scores (**Table S4**) at 30-minute intervals during the HDM exposure. Each of the symptom score components was recorded on a Likert-type scale ranging from 0 to 3 (**Table S4**). In total, 23 participants completed the 5-hour HDM exposure in the ACC; one participant in group 1 failed FEV1 criteria (outlined below) during the exposure and was removed from the ACC (**Figure S1A**).

C.1.b Safety: monitoring FEV1 in the ACC for HDM-PARC+AA+ study

Subjects experiencing a decrease in FEV1 $\geq 12\%$ during the HDM exposure in the ACC were removed from the exposure chamber into a clean room devoid of antigen for an

observation period. Treatment with albuterol, by MDI or nebulizer, was provided and the subject was returned to the ACC. A total of six participants met this criterion (**Figure 3C**). Per protocol, subjects experiencing a second decrease in FEV1 $\geq 12\%$ after being returned to the ACC were properly treated as described above and discontinued from the study for safety precautions. Only one participant met this criterion (**Figure S1A**) and was thus not included in the analyses. Per protocol, subjects experiencing an initial 50% or greater decrease in FEV1 would be removed from the exposure chamber, treated, and enter an observation period. These subjects would then be discontinued from the study for safety precautions; none of the study participants met these criteria.

C.1.c Der p 1 levels

The Der p 1 content in the delivered HDM body powder (Lot# RMB82M (18950)) was approximately 249.04 and 231.97 ng/M³ in HDM-PARC+AA+ study groups 1 and group 2, respectively, during the 5-hour HDM exposure (**Table S12**). As described above, collector filters were placed at three workstations in the seating area in the ACC. Antigen delivery rates and dispersal patterns were adjusted to maintain equivalent antigen levels throughout the chamber and in both study groups.

C.1.d Readouts of disease severity

The readouts/metrics of disease severity used in the study are outlined in **Figure 1C-D**, **Figure S1C**, **Table S1**, and **Table S4**. The metrics are further described below.

Total Symptom Scores (TSS) reflect the sum of total nasal symptom scores (TNSS; nasal congestion, sneezing, runny nose, and nasal itching) and total ocular symptom scores (TOSS; itching, redness, and tearing) (**Table S4**). TSS was scored on a Likert-type scale ranging from 0 to 3 and ranged from 0 to 21.

Summated Symptom Scores (SSS) reflect the sum of TNSS, TOSS, and the total asthma symptom score (TASS; cough, wheeze, and dyspnea). The SSS ranges from 0 to 30 (**Table S4**). SSS is a disease severity metric that was assessed in cohort 6 participants.

FEV1: The forced expiratory volume of air in one second (FEV1) was measured by spirometry and was used as a measure of lung function. FEV1 is a disease severity readout that was measured in cohort 6 participants.

ACQ-7: The Asthma Control Questionnaire (ACQ)-7 is a 7-item questionnaire reflective of one-week prior to assessment and was used as a measure of the adequacy of asthma control⁶. The ACQ-7 reflects the average score from the summated response to six questions and pre-bronchodilator FEV1 and is a disease severity metric that was measured in cohort 6 participants.

Instantaneous vs. Reflective symptom assessment: Symptom scores were recorded in the ACC instantaneously (designated as i= instantaneous; e.g., iSSS) at 30-minute intervals. Symptom scores were also recorded in the natural environment (pre and post

ACC) in subject symptoms diaries; in this context, participants were instructed to reflect upon and score symptoms twice daily (AM and PM; designated as r= reflective, e.g., rSSS). Reflective symptoms were recorded four days prior to the ACC (Run-In; study days -4 to -1) and four days after the ACC (study days 1 to 4) in the natural setting (Figure 1D).

C.1.e Study visit descriptions for HDM-PARC+AA+ study

The study consisted of five study visits. A description of each of the visits and related activities conducted are outlined below.

Visit 1 (Screening Visit; days -21 to -5)

Subjects provided informed consent and a series of assessments and procedures were performed, including spirometry, skin prick testing, and obtaining medical history to determine eligibility to participate in the study.

If spirometric criteria were met, blood and nasal brushings were obtained. A Medical Event Calendar (MEC) and a Subject Symptoms Diary were dispensed to each subject to monitor adverse events, concomitant medications, and symptoms. During the Run-in Period (study days -4 to -1), subjects were instructed to assess and record use of rescue albuterol medication and night-time awakening on the MEC. Reflective TNSS, TOSS, and TASS were scored in the AM and PM. Subjects were also given handheld spirometric devices (Vitalograph asma-1TM) to record FEV1 (AM and PM).

Visit 2 (ACC HDM exposure Visit; day 0)

Upon confirmation of continued eligibility criteria being met, all pre-chamber procedures being completed, and ACQ-7 obtained, subjects entered the ACC. Nasal brushings were performed and blood was drawn prior to entry into the ACC. Chamber Visit Diaries for symptom and FEV1 recordings during the HDM exposure were dispensed to all subjects with instructions for completion. Disease metrics were recorded at 30-minute intervals until completion of the exposure. Spirometry and pulse oximetry were performed at baseline and at 30-minute intervals until completion of the exposure.

The total Chamber Visit exposure time from baseline to end was five hours. Exposures commenced at 8:00 A.M. All study procedures, including antigen collection and symptoms assessments, were as outlined. Time out of the Chamber was minimized as much as possible. Halfway through the exposure (at hour 2.5), subjects were removed briefly for a nasal brushing and immediately returned to the ACC. Prior to subjects being discharged, at the end of the exposure, nasal brushings were performed and blood was drawn. Subjects were issued materials (MEC, Subject Symptoms Diary, and spirometric device) with instructions and were scheduled for the Late-phase response Visit (Visit 3).

Visit 3 (Late-phase response; day 1)

Visit 3 occurred 24 hours after Visit 2. The MEC, Subject Symptoms Diary, spirometric recordings, adverse events (AEs), and concomitant medication use were reviewed and recorded. Subjects assessed symptoms and spirometry and pulse oximetry were

obtained. Subjects were issued a MEC, Subject Symptoms Diary, and spirometric device with instructions and scheduled for the following visit (Visit 4).

Visit 4 (Follow-up Visit; day 5) was conducted exactly as the Late-phase response visit but also included the completion of an ACQ-7.

Visit 5 (Follow-up Visit; day 31) was conducted exactly as Follow-up 1 visit. No materials were dispensed as this was the final study visit.

C.1.f Der p 1 levels delivered into the ACC for HDM-PARC+AA+ study

The delivered HDM powder was collected on a nylon filter (Indoor Biotechnologies, Inc., Charlottesville, VA) and a silicone-greased slide via an Allergenco-D Posi-track disposable cassette (Environmental Monitoring Systems, Inc, Charleston, SC) with suction pumps operating at 15L of air sampled/minute for a 10-minute interval. There were 3 collection stations and samples were collected at 10, 60, 120, 180, 240, and 300 minutes after initiating dispersal of the HDM milled powder into the ACC. The nylon filters collected in the ACC were analyzed for Der p 1 levels by ELISA. Filters were transferred to 50 mL conical tubes, washed with 1 mL of PBS-tween²⁰ (0.01%), vigorously vortexed for one minute, and then centrifuged at 3000 rpms for 10 minutes. An ELISA kit was used to assay Der p 1 levels (EL-DP1; Indoor biotechnologies), following the manufacturer's instructions.

C.1.g LPS content in HDM delivered in the ACC

The HDM used in this current study is from the same batch used in our previous study⁵. This batch of HDM has been previously tested by us for LPS content (Lonza kit) as previously described⁵. The kit that we used was the chromogenic Lylulus amebocyte lysate (LAL) assay. In our previous study using this batch of HDM extract, the level of LPS delivered during a period of 10 minutes in the ACC was 421.51 ng/m³ or in endotoxin units (EU): 42.2 EU/m³. The amount of LPS extracted/obtained from 1mg of HDM in 1mL of solution was 149.86 ng/mL or 14.98 EU/mL.

C.2. Hierarchical clustering

Unsupervised hierarchical clustering with Ward's D linkage and Manhattan distances were used to derive evoked phenotypes [resilient (R), adaptive (A), and maladaptive (M)]: 11 iSSS measures recorded at 30-minute intervals during the ACC were used. To derive CD8-CD4 balance clusters, unsupervised clustering was performed with baseline peripheral blood CD8+ T-cell counts and CD4:CD8 T-cell ratio values derived by flow cytometry (methods detailed below) using Euclidean distances and Ward's D linkage. Association of T-cell traits with phenotypes was examined by performing unsupervised clustering of significant T-cell traits [false discovery rate (FDR)<0.05] using Ward's D linkage and Euclidean distances and supervised clustering of participants by phenotype.

C.3. iSSS-AUC, FEV1-AUC, iTSS-AUC, and iTASS-AUC calculation

To represent overall disease severity by a single metric, we used the area-under-the-curve (AUC) statistic. For calculating iSSS-AUC, the iSSS vs. time (hours) line was

plotted for each participant, and the AUC was calculated for each participant using the trapezoid method and then divided by 5 (hours) to rescale the value into the same range as the iSSS. The same methods were used to derive the iTSS-AUC, iTASS-AUC, and FEV1-AUC.

C.4. Immunophenotyping: HDM-PARC+AA+ study

C.4.1. Serum HDM-specific IgE

Serum HDM-specific IgE (sIgE) was evaluated by ImmunoCAP assay by ThermoFisher as described previously⁴.

C.4.2. Flow cytometry

Heparin collected peripheral blood was collected from the HDM-PARC+AA+ participants (100 μ L) and stained for 20 minutes at room temperature by the addition of the antibody master mix for the corresponding antibody panel (**Table S5**). Subsequently, blood was lysed with 1X BD FACS lysing solution (BD Biosciences) following the manufacturer's instructions, centrifuged and washed twice with FACS buffer. Cells were re-suspended in FACS buffer and data was acquired in a BD Fortessa and was analyzed using FlowJo v10.6 (BD Biosciences) as described previously⁴. All cell counts were log₂-transformed for analysis.

C.5. Peripheral blood trait strata for HDM-PARC+AA+ study

Four peripheral blood traits were evaluated as categorical variables. The cutoffs dictating whether an individual was classified as higher vs. lower for each of the traits are described below. Cell counts were derived from whole blood flow cytometry and serum HDM-specific IgE (sIgE) levels by ImmunoCAP.

- CD8-CD4^{hi}: Refers to the CD8-CD4 balance cluster that had higher CD4+ and CD8+ T-cell counts (**Figure 5A**).
- Eosinophil^{hi} cut-off was >150 cells/ μ L. This cutoff has been used for initiating anti-eosinophilic therapies and as a biomarker in patients with moderate to severe eosinophilic asthma⁷⁻⁹.
- sIgE^{higher} cut-off was 0.35 kU/L (Ref.¹⁰) This cut-off was used to discriminate lower vs. higher sIgE levels.
- Neutrophils^{hi}: Neut^{hi} was > 1×10^6 cells/mL. This cutoff approximated the median baseline neutrophil levels in HDM-PARC+AA+ cohort: 1.045×10^6 cells/mL.

C.6. Flow cytometry analysis for HDM-PARC+AA+ study

Cell counts (cells/mL) were log₂-transformed for statistical analysis. To examine overall differences in levels of a cell type over the exposure, a linear GEE model was used, based on the normal distribution and adjusted by time (pre- and post-ACC) with an exchangeable correlation structure. When comparing phenotypes, an ANOVA was applied to test for if there is a difference between at least 2 phenotypes. An FDR was used to correct for multiple comparisons within each of the three panels evaluated

(**Tables S8**, and **S9**). An FDR<0.05 was used to classify a trait as significant. Traits that were identified as significant by phenotypes (total, 24 traits; **Table S8**) were included in a heatmap (**Figure S10**). The average value was used in the heatmap and z-score transformed (mean centered then divided by standard deviation) within each row (trait). Unsupervised clustering was performed on the row (traits) using Ward's D linkage and Euclidean distances. Supervised hierarchical clustering was performed on the column (HDM-PARC+AA+ participants) by evoked phenotype.

C.7. Nasal cell sample collection and RNA isolation for HDM-PARC+AA+ study

Nasal cell sample collection and RNA isolation was performed as described by us previously with minor modifications^{4,11}. Briefly, RNase- and DNase-free 4N6FLOQSwabs™ (Life Technologies) were used for nasal brushings. Nasal cells were collected by brushing with a sterile swab inside the nostril using circular movements against the inferior turbinates. Subsequently, swabs were placed into a microcentrifuge tube containing RNA lysis solution (Buffer RLT, Qiagen) supplemented with β -mercaptoethanol (1:100) (Sigma). Samples were immediately refrigerated, stored at 4°C overnight and RNA was isolated the next day. If the collected swab showed evidence of hemorrhage, the sample was excluded. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase I digestion according to the manufacturer's instructions. RNA quantity and purity were determined by qubit assay and spectrophotometry (260/280=1.8-2.0) (Nanodrop) respectively and integrity (RIN) was determined using an Agilent 2100 Bioanalyzer with an RNA 6000 Nano assay (Agilent Technologies, Palo Alto, CA). Samples with RIN ≥ 7.0 were selected for RNA

sequencing (RNA-Seq). A total of 23 HDM-PARC+AA+ participants completed the HDM exposure study and had nasal brushings collected at t=0, 2.5, and 5 hours of HDM exposure (**Figure 1D**), of which 20, 22, and 21 samples, respectively, passed the quality control criteria and were used for RNA-sequencing.

C.8. Library preparation and sequencing for HDM-PARC+AA+ study

Psomagen performed library preparation using the TruSeq Stranded mRNA kit and sequencing (2 x 151 bp paired end on a NovaSeq6000 using s4 chemistry). Sequences were aligned and mapped to the UCSC hg38 genome (Illumina igenomes) using hisat2 v2.1.0¹² and counts for 26,485 genes were obtained using HTSeq v0.11.2¹³.

C.9. RNA-Seq data filtering and normalization for HDM-PARC+AA+ study

Gene counts were normalized, and dispersion values were estimated using the R package, DESeq v1.34.0. Prior to filtering the dataset there were a total of 26,485 genes. As described previously⁴, genes with a gene count of 0 across all samples were removed, resulting in 23,896 genes), and the remaining 0's were changed to 1's. The size factors were estimated using the gene expression and used to normalize the gene-counts. Genes whose expression levels were below the 25% in terms of expression were removed. After filtering, the total number of genes used for downstream analysis were 17,920.

C.10. Gene signature derivation (z-scores)

To generate the asthma^{up} z-score, the log₂-normalized expression of each gene within the gene signature was z-transformed (mean centered then divided by standard deviation) across all samples and then averaged. The 18 genes comprising the asthma^{up} gene signature are outlined below and were identified as upregulated in asthmatic nasal cell transcriptomes compared to those of healthy controls [Figure 4 of Ref.¹⁴].

<i>CCL26</i>	<i>CST4</i>	<i>PRSS33</i>
<i>CDH26</i>	<i>DPP4</i>	<i>PXDN</i>
<i>CLC</i>	<i>IL1RL1</i>	<i>SERPINB2</i>
<i>CLCA1</i>	<i>IL4</i>	<i>SLC7A1</i>
<i>CPA3</i>	<i>MS4A2</i>	<i>TPSAB1</i>
<i>CST1</i>	<i>POSTN</i>	<i>WSCR17</i>

D. Statistical analyses (cohorts 1-6)

Detailed statistical analyses and approaches are described outlined in the figure legends and **Table S2**. For each immune cell trait analyzed by flow cytometry, cell trait values of 0 were imputed to be ½ the lowest observed non-zero value within that cell trait. Cells traits in which 50% or more had a value of 0 were excluded from analysis. Flow cytometry data was log₂ transformed for all analyses. Briefly, statistical analyses of clinical and immunophenotyping data included paired t-test, Fisher's exact test, z-transformation, linear regression, linear generalized estimating equation (GEE) models with an exchangeable correlation structure and adjusted by time as a factorial variable, likelihood ratio test (LRT), and analysis of variance (ANOVA). An FDR <0.05 was used to identify significant traits associated with the evoked phenotypes. For longitudinal analysis of gene expression data, GEE-generalized linear model (GLM) models based

490 on the normal distribution (z-scores) and gamma distribution (single genes) were used.
491 Models were adjusted by time and had an autoregressive (1) correlation structure;
492 significance was tested by ANOVA. For cross-sectional comparisons, negative binomial
493 GLM implemented in DESeq (single genes) and a linear model (z-scores) was used,
494 and statistical significance was tested by LRT.

Supplementary Notes

Supplementary Note 1. Aeroallergen challenge chamber operations

Supplementary Note 1a. Overview

The aeroallergen challenge chamber (ACC) used in each of the challenges for cohorts 1-6 presented in this study was developed with the objective of providing a highly controlled environment that mitigates confounders and can be used to evaluate allergic responses to allergen exposures in pharmacologic, mechanistic, and clinical trial studies. Details of the ACC and its operations are as previously reported by us^{2,4,5,11,15-17} and are described below and illustrated (**Figure below**).

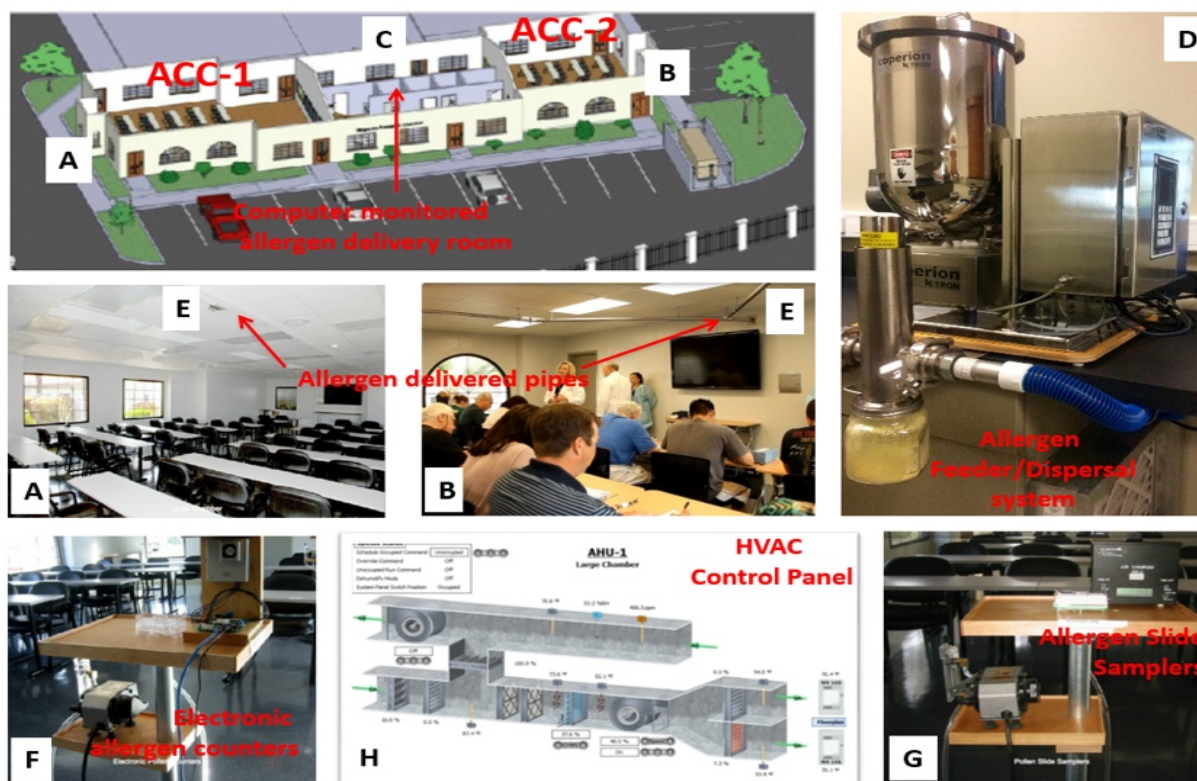


Figure. Illustration of Aeroallergen Challenge Chambers at the Biogenics Research Chamber. (A) Larger ACC (10,846 ft³) **(B)** Smaller ACC (6,655 ft³) **(C)** Central room housing the computer-controlled feeder/antigen-dispersal system. **(D)** Feeder/antigen-dispersal system. **(E)** Pipes used for routing antigen from the feeder to the ACC seating area. **(F-G)** Stations for collecting and dispersed antigen in the ACC. **(H)** Highly standardized Heating Ventilation Air Conditioning system used to control the temperature and humidity in the ACCs.

Supplementary Note 1b. Operations

The Biogenics Research Chamber, site where ACC studies are conducted, houses two ACCs with a central control room for antigen delivery. The larger ACC can seat up to 50 persons and the smaller ACC can seat up to 12 persons. Each of the ACCs has the ability to achieve the uniform dispersal of allergens throughout the chambers without the use of fans which produce air currents in the direction of study subjects that could adversely affect allergic (nasal and ocular) symptoms. Each ACC, with a central control room and staging area, was built using clean room technology. Chairs and tables within the ACC are movable to allow for ease in cleaning and for different configurations for different protocols. Audio and video systems are used for communication between the chamber and the control room. There are viewing windows for monitoring from the control room into the chamber. The air-handling system is independently computer-controlled to adjust for the occupancy of the chamber. In testing mode, fresh outside air is drawn through highly filtered intakes, chilled in a single pass across the condenser, and distributed from a duct system by powered, filtered diffusers from the ceiling and exhausted by powered, filtered vents at the floor level to the outside environment air exchange rates are 12 full cycles per hour. This system delivers a slightly positive pressure inside the chamber to reduce the introduction of outside contaminants with opening of doors. When switched to an unoccupied mode, the system functions as standard air-conditioning recirculating the air. An alternate electrical source is a diesel-powered generator with sufficient capacity to power the chamber in full operational mode at maximum outside temperatures. The computer monitors the status of all filters, diffusers, and exhausts.

542

543 The ACC operations for all cohorts 1-6 presented in this study were the same as in our
544 previous studies^{2,4,5,11,15-17}. Briefly, the water-chilled air conditioning unit was adjusted to
545 maintain temperature and relative humidity at 70-72°F and ~50%, respectively. Air
546 entered the chamber via powered HEPA-filtered registers that maintain constant airflow.
547 When airflow, temperature, and humidity reached stable conditions, the antigen
548 dispensing system was activated. The dispersal systems were as previously
549 reported^{2,4,5,11,15-17}. Briefly, the antigen for all studies was delivered by a computer-
550 controlled feeder that delivers an adjustable weight of allergen in grams per hour into a
551 vortex driven by an adjustable blower augmented by an eductor specially designed for
552 this purpose. The respective antigen for each of the studies was dispersed from feeder
553 into a series of grounded stainless-steel tubes traversing the ceiling above the subject's
554 seating areas in the ACC. These tubes are designed with specially bored ports and
555 specially machined joints locked by hand-cranked clamps that allow for directional
556 adjustment of allergen. Throughout each of the challenges, antigen counts were
557 performed using 2 different manual Allergenco systems, MK-3 staged counters and
558 Allergenco-D Posi-track disposable cassettes which are powered by suction pumps run
559 at 15 L/min. The Allergenco systems are mounted on a platform at 46-in from floor level.

560

561

Supplementary Note 1c. COVID-19 adaptations

Month and year of each of the challenge studies are reported (**Table S1**). The cohort 3 challenge study is the only study presented here that was conducted during the Coronavirus 2019 (COVID-19) pandemic. Study participants and staff underwent COVID-19 testing (Becton-Dickinson Veritor™ System for Rapid Detection) at the screening visit within 24 hours of entry into the ACC. All participants entered the ACC with protective masks and were seated more than 6 feet apart in all directions with sneeze guards on 3 sides of each seat (**Figure below**). Masks were removed upon activation of the pollen feeder in the ACC. Further evaluation of the COVID-19 adjusted protocols are being performed and are the primary subject of a separate manuscript under review. Tables and seats within the ACC were arranged for 6 feet social distancing. Plexiglass sneeze-guards were added on 3 sides of each seat. Two series of pollen collections and counts were done, 30 minutes apart, utilizing the Allergenco cassette system within 12 of 25 plexiglass sneeze-guard areas and in the 4 corners and center of the open chamber. Pollen counts at all sites, including inside 12 sneeze-guards, were within the specified limits demonstrating good and equal distribution.



Figure. Modifications of ACC setup during the COVID-19 pandemic. Birch pollen was dispersed ($3,500 \pm 750$ grains/m³) from a series of tubes along ceiling into the ACC. Two series of pollen collections and counts were done, 30 minutes apart, utilizing the Allergenco cassette system within 12 of 25 plexiglass sneeze-guard areas and in the 4 corners and center of the open chamber. Pollen counts at all sites, including inside the 12 tested sneeze-guards, were within the specified limits demonstrating good and equal antigen distribution.

Supplementary Note 2. Polysensitization and sIgE in Meta-Analysis

We conducted a meta-analysis of cohorts 1 (Virginia live oak), 2 (Mountain cedar), and 4 (Timothy grass). These cohorts were selected because they had the same study design (2 consecutive exposures of 3 hours/day) and the same symptom severity metric recorded during the exposures (iTSS; **Figure 1C**). For the meta-analysis clustering, all 14 iTSS participant-recorded measurements were used to derive evoked phenotypes (**Figure 2G**). We tested whether polysensitization status and/or IgE levels specific to the aeroallergen in each cohort associated with the evoked phenotypes. The approach to perform this analysis was as follows.

Polysensitization status was defined by the total number of positive skin prick tests (SPTs) (≥ 5 mm reactivity to aeroallergen compared to saline control). This analysis was performed using a group of pollen SPTs common to all three cohorts in the meta-analysis. A SPT for Timothy grass was completed for cohort 4, but not cohorts 1 and 3; thus, it was excluded. Due to exclusion of Timothy grass, inclusion of the SPTs for Virginia live oak and Mountain cedar would have resulted in a bias and were thus excluded as well. The SPTs included in the analysis were ragweed, cedar elm, pecan, Arizona ash, spiny pigweed, Bermuda grass, and Johnson grass. Because this is SPT count data where the mean (2.38) and variance (4.24) were not equal, the GLM based on the negative binomial implemented in the R package MASS¹⁸ was used (opposed to the GLM based on the Poisson distribution), in conjunction with the likelihood ratio test (LRT). The cohort variable significantly associated with SPT ($P < 0.001$) and thus, the model testing the association of polysensitization status and evoked phenotypes was

adjusted by cohort. After adjusting for cohort, evoked phenotypes did not associate with polysensitization status ($P=0.254$). Using the coefficients for cohort from the model, an SPT adjusted by cohort was derived and plotted by evoked phenotypes (**Figure 2H**).

The levels of sIgE (determined by the level of seasonal aeroallergen-specific IgE (sa-sIgE) used in the respective cohort) were commercially acquired. Study participants were defined as having high sa-sIgE levels (ss-algE^{hi}) if sa-sIgE levels ≥ 0.35 kU/L, otherwise they were defined as having low sa-algE levels (sa-IgE^{lo}). The cohort variable was also associated with sa-sIgE^{hi/lo} ($P=0.019$; Fisher's exact test) with 32.4% ($n=11$) of cohort 1 having sa-sIgE^{hi}, 76.1% ($n=15$) of cohort 2 having sa-sIgE^{hi}, and 50.0% ($n=11$) of cohort 4 having sa-sIgE^{hi}. A logistic regression model with a LRT was then used to test the association between sa-sIgE^{hi/lo} and evoked phenotype while adjusting for cohort. After adjusting for cohort, evoked phenotype was associated with sa-sIgE^{hi/lo} ($P=0.004$). To graphically represent this, a bar plot was used as opposed to plotting the odds ratio with 95% confidence intervals due to the fact that the maladaptive evoked phenotype had no participant who classified to sa-sIgE^{lower}, resulting in the 95% CI being 0 to infinity.

Supplementary Note 3. CD8+ T-cells: role in ARC/AA

Additional studies in humans, although limited, have also described a role of CD8+ T-cells in asthma. Levels of bronchial CD8+ T-cells have been associated with declining lung function¹⁹⁻²¹. Akin to findings in murine studies, subsets of CD8+ T-cells overrepresented in airways and peripheral blood of persons with asthma have been identified to be additional sources of type 2 cytokines^{22,23}. In a recent study, single-cell RNA-seq analysis of bronchoalveolar lavage fluid identified an enrichment of a CD8+ T-cell cluster in persons with asthma compared to healthy controls²⁴. Postmortem examination of lung tissues from patients who died from asthma had higher levels of CD8+ T-cells compared to individuals without lung disease²⁵. Supporting our findings (**Figure 6**), expansion of CD8+CD28- T-cells was identified in induced sputum of persons with severe asthma compared to those with mild asthma and healthy controls²⁶. Additionally, circulating CD8+ T-cells of persons with severe asthma manifest an activated transcriptomic profile compared to those with non-severe asthma²⁷.

Supplementary Note 4. Limitations

We acknowledge that our inclusion/exclusion criteria omits persons with GINA-classified severe AA. However, in this patient population, these challenge studies would be extremely difficult to undergo not only because of the safety concerns of challenging persons with severe AA with disease triggers but also because per ACC protocols, study participants are required to withhold medications for four days leading up to the day of challenge. Thus, persons with severe AA would likely be unable to satisfy this criterion; if we omitted this criteria, our results (i.e., symptoms experience by these participants in the ACC) would likely be attenuated and confounded by treatments. However, our previous work demonstrates parallels in the mechanisms mediating resistance to disease severity in non-atopic non-allergic controls⁴ and persons with mild/resilient ARC without asthma¹¹. In line with these findings, as a proxy for severe AA, we can identify and study persons with mild-to-moderate AA who manifest maladaptive responses to disease triggers. In doing so, we can potentially gain a better understanding of the mechanisms underpinning maladaptive disease in persons with severe AA.

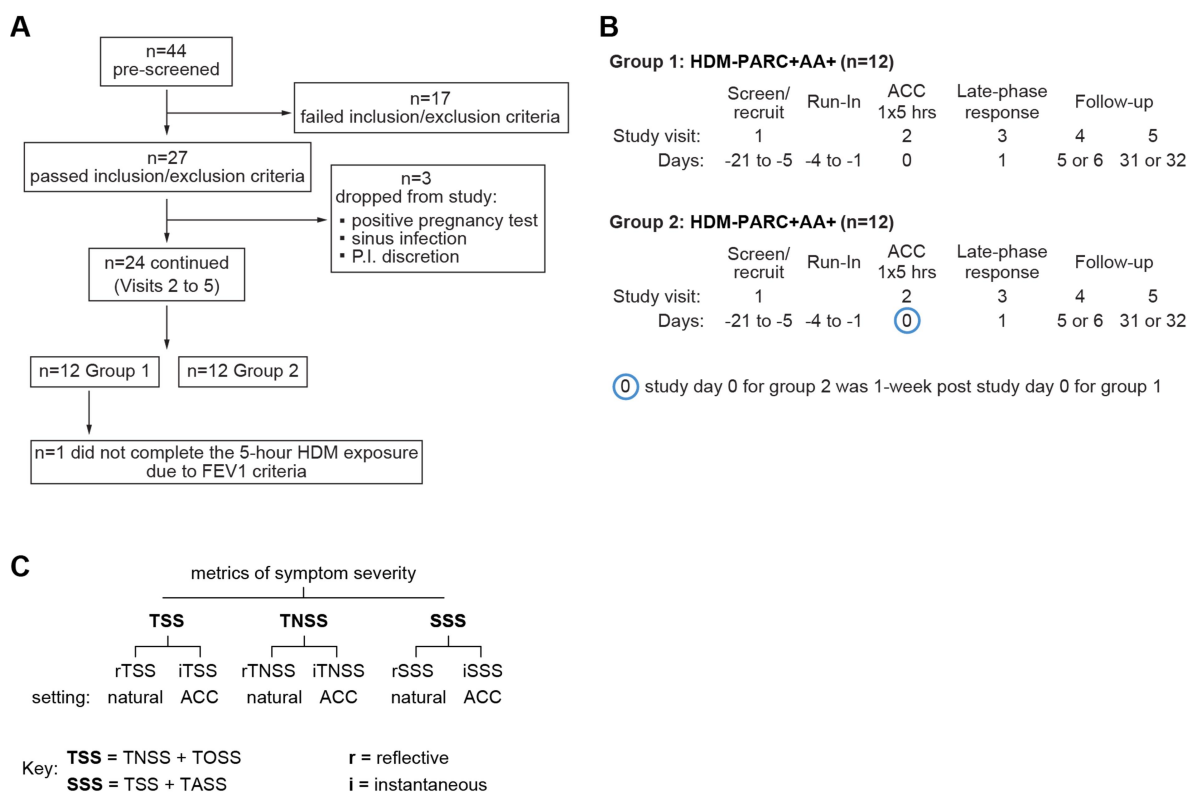
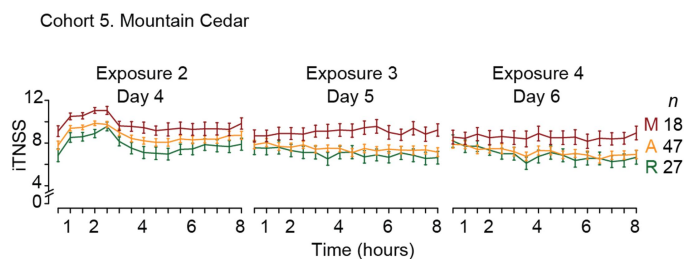
666 **Supplementary Figures**

Figure S1. HDM-PARC+AA+ Study Criteria and Design and symptom severity metrics used in this study. (A) Screening and recruitment of HDM-PARC+AA+ (cohort 6) participants. **(B)** HDM-PARC+AA+ (cohort 6) study design. The ACC used in this study seats a maximum of n=12 participants; thus, the 24 study participants were divided into 2 groups (group 1 and group 2) that completed the same study regimen 1 week apart. **(C)** Symptom severity metrics used.



676

677 **Figure S2. Symptom severity in evoked phenotypes (cohort 5).** Mean (SEM) iTNSS

678 at 30-minute intervals in cohort 5 participants during Mountain cedar exposure days 2,

679 3, and 4 by phenotypes (resilient [R], adaptive [A], maladaptive [M]). For exposure days

680 2-4, symptom recordings began 30 minutes after initiation of aeroallergen exposure in

681 the ACC.

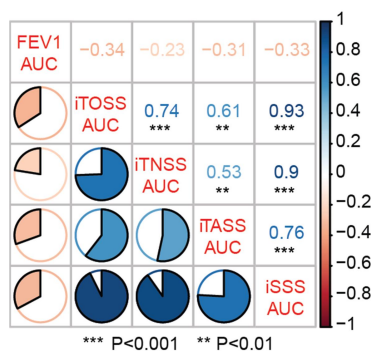
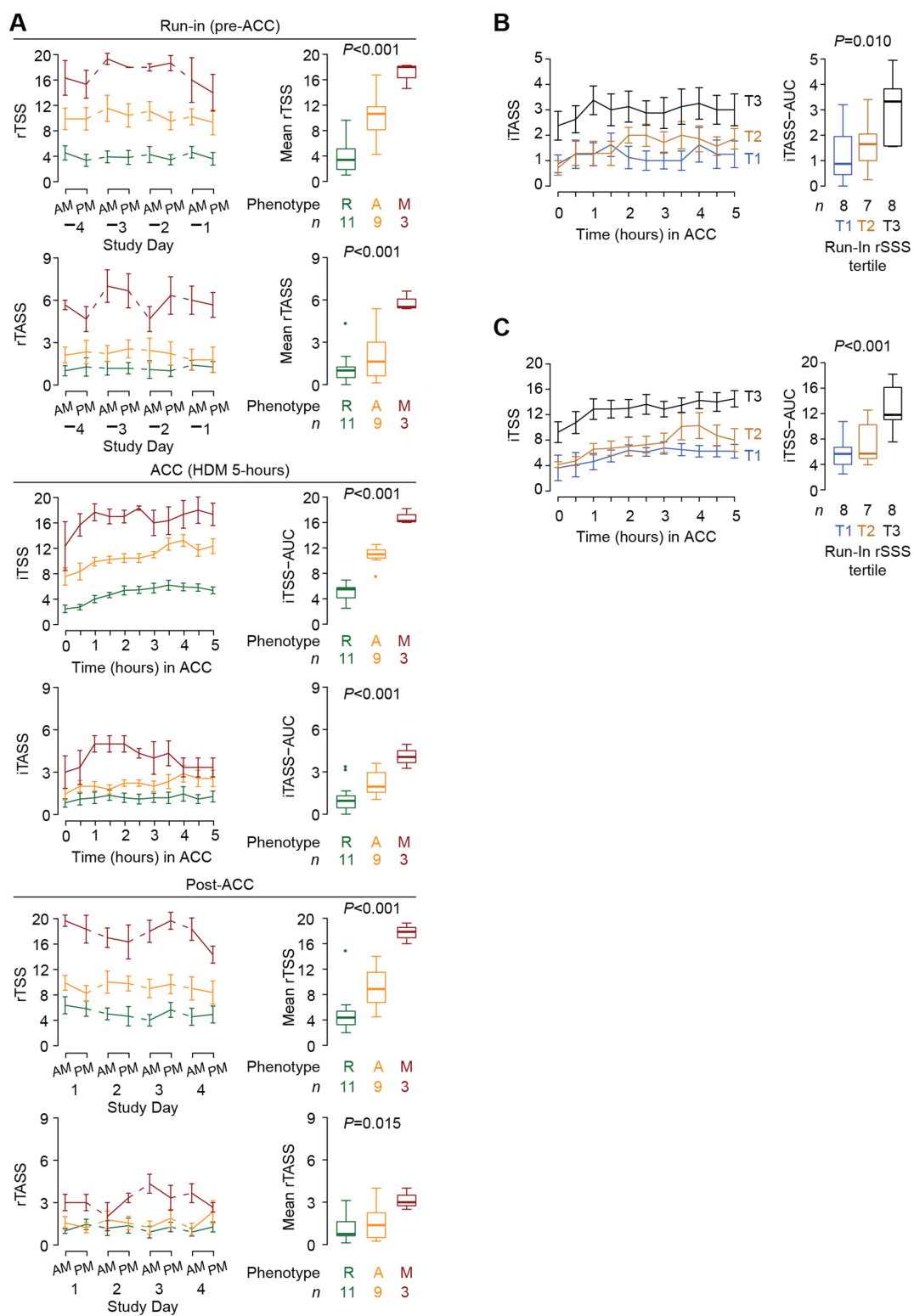


Figure S3. Correlation of FEV1 and symptom scores. Pearson's correlation coefficient and pie chart illustrating Pearson's correlation coefficient to demonstrate the associations of FEV1 and disease severity metrics. *P* determined by Fisher's z-transformation.

687 **Figure S4.**

689 **Figure S4. Symptom severity in HDM-PARC+AA+ evoked and constitutive**
 690 **phenotypes. (A)** Rows 1-2: *Left*, Mean (SEM) reflective total symptom score (rTSS)
 691 and reflective total asthma symptom score (rTASS) at the indicated timepoints, and
 692 *Right*, Mean rTSS and rTASS in the 4-day run-in by HDM-PARC+AA+ phenotypes
 693 (resilient [R], adaptive [A], maladaptive [M]). Rows 3-4: *Left*, Mean (SEM) instantaneous
 694 (iTSS) and iTASS at the indicated timepoints, and *Right*, iTSS-AUC and iTASS-AUC in
 695 the ACC by HDM-PARC+AA+ phenotypes. Rows 5-6: *Left*, Mean (SEM) rTSS and
 696 rTASS at the indicated timepoints, and *Right*, Mean rTSS and rTASS in the natural
 697 setting by HDM-PARC+AA+ phenotypes. *P*, by LRT. **(B)** *Left*, Mean (SEM) iTASS at the
 698 indicated timepoints. *Right*, iTASS-AUC in the ACC by HDM-PARC+AA+ run-in rSSS
 699 tertiles [tertile 3 (T3) highest tertile]. **(C)** *Left*, Mean (SEM) iTSS at the indicated
 700 timepoints. *Right*, iTSS-AUC in the ACC by HDM-PARC+AA+ run-in rSSS tertiles. **(B-C)**
 701 *P*, by LRT.
 702

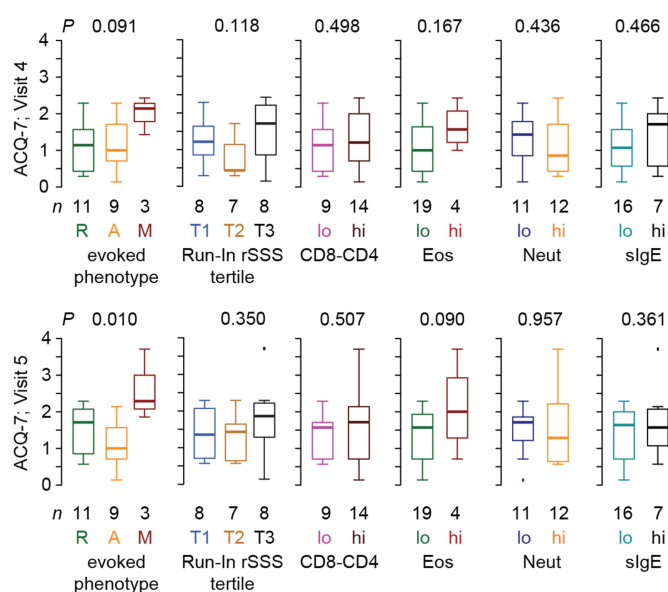


Figure S5. Asthma control questionnaire (ACQ)-7 scores. Mean asthma control questionnaire-7 (ACQ-7) scores at visits 4, and 5 in HDM-PARC+AA+ participants (cohort 6) stratified by (*left to right*) evoked phenotypes, Run-in rSSS tertiles [tertile 3 (T3) highest tertile], and CD8-CD4, eosinophil (Eos), neutrophil (Neut), and HDM-specific IgE (slgE) strata. *P*, by LRT. Hi, higher; Lo, lower.

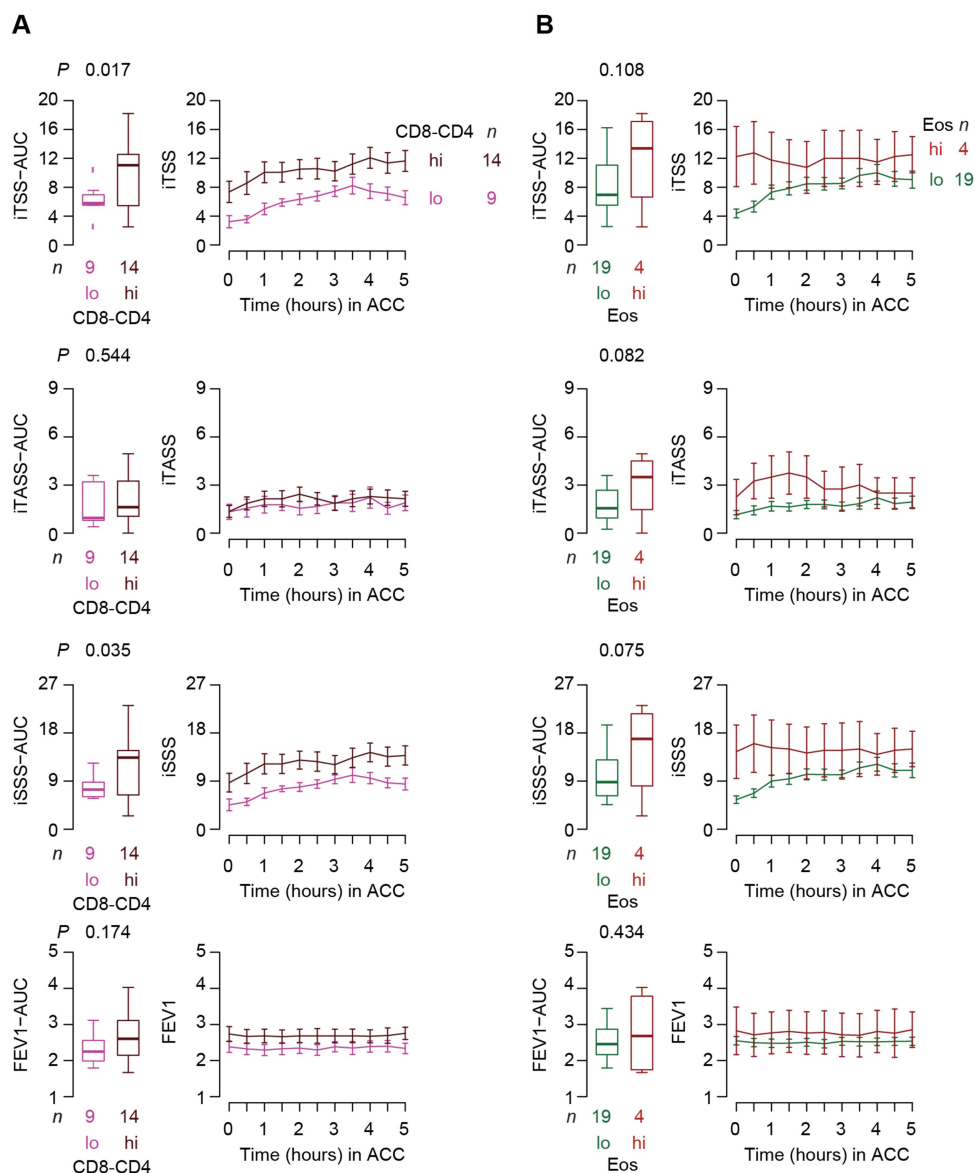
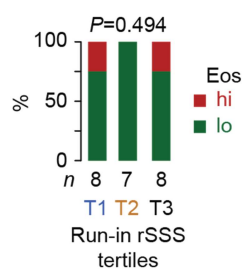


Figure S6. Disease severity in the ACC of HDM-PARC+AA+ participants stratified by peripheral blood trait strata. Top to bottom: iTSS-AUC and iTSS, iTASS-AUC and iTASS, iSSS-AUC and iSSS, and FEV1-AUC and FEV1 stratified by (A) CD8-CD4 balance clusters (CD8-CD4^{hi} vs. CD8-CD4^{lo}; left column) and (B) eosinophil (Eos^{hi} vs. Eos^{lo}; right column) strata. Line plots are depicted as mean (SEM). *P* values determined by linear model with LRT for comparing the indicated strata.



717

718 **Figure S7. Eosinophil strata by Run-In rSSS tertiles.** Proportion of Run-In rSSS
 719 tertiles [tertile 3 (T3) highest tertile; predictor] with the indicated eosinophil (Eos)^{hi} vs.
 720 Eos^{lo} strata. *P*, by Fisher's exact test. Hi, higher; Lo, lower.

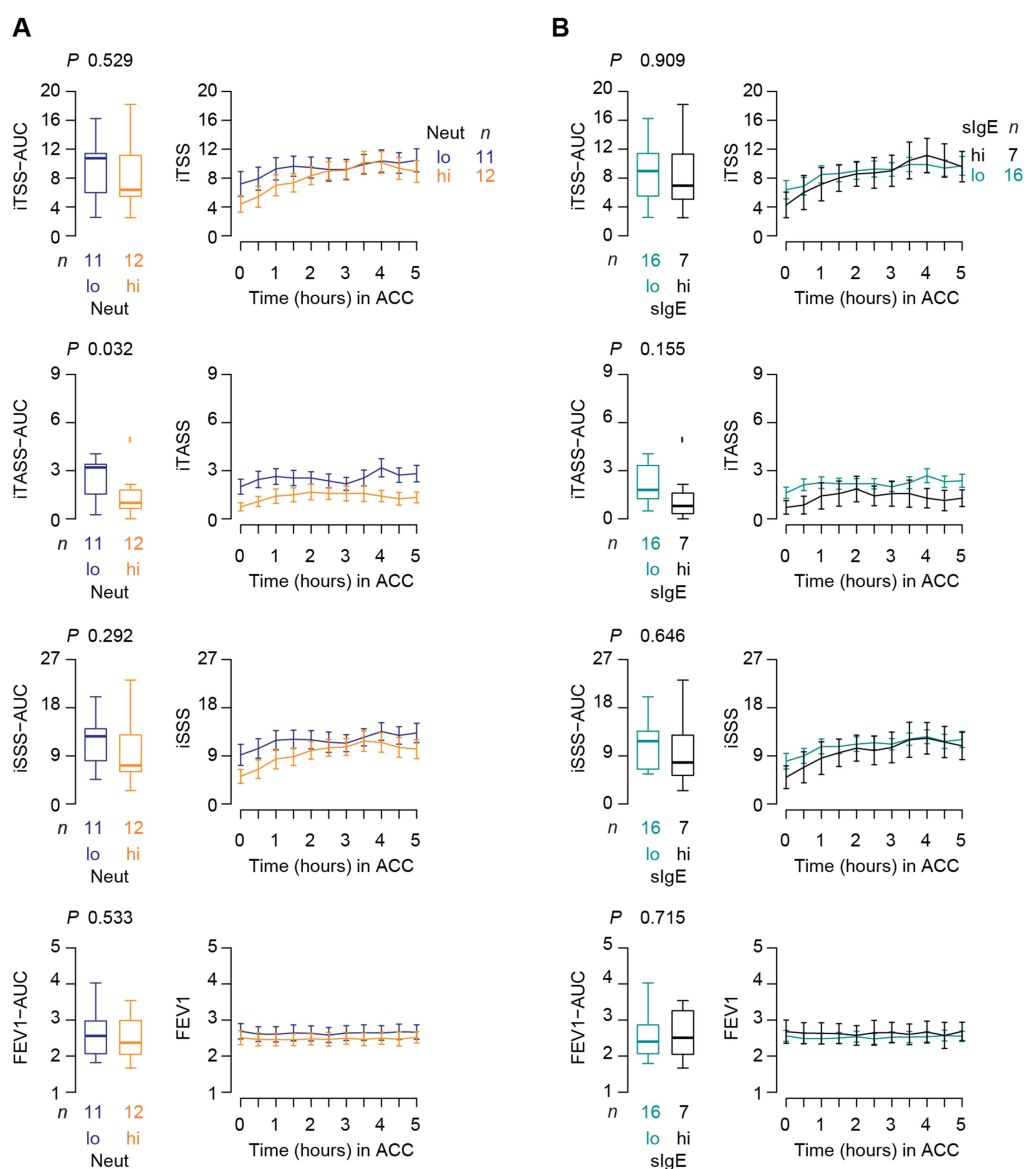


Figure S8. Disease severity in the ACC of HDM-PARC+AA+ participants stratified by peripheral blood trait strata. Top to bottom: iTSS-AUC and iTSS, iTASS-AUC and iTASS, iSSS-AUC and iSSS, and FEV1-AUC and FEV1 stratified by (A) neutrophil (Neut^{hi} vs. Neut^{lo}) and (B) slgE (slgE^{hi} vs. slgE^{lo}) strata. Line plots are depicted as mean (SEM). P values determined by linear model with LRT for comparing the indicated strata.

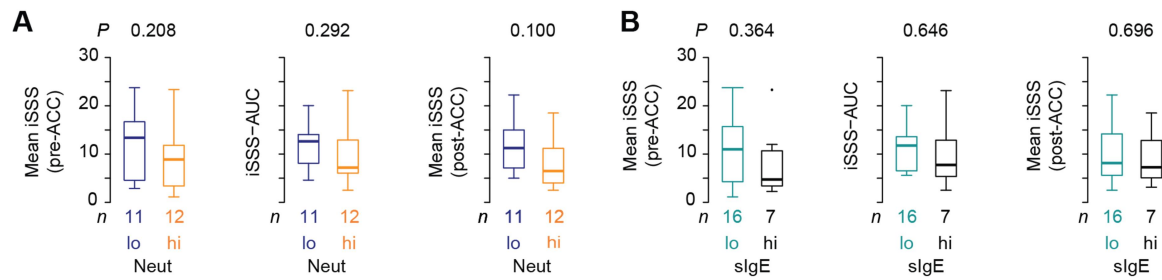


Figure S9. Disease severity before, during, and after the ACC of HDM-PARC+AA+ participants stratified by peripheral blood trait strata. Mean iSSS in the 4-day run-in and natural settings (pre and post ACC), and iSSS-AUC in the ACC in HDM-PARC+AA+ participants stratified by **(A)** neutrophil (Neut^{hi} vs. Neut^{lo}), and **(B)** sIgE (sIgE^{hi} vs. sIgE^{lo}) strata. *P* values determined by linear model with LRT for comparing the indicated strata.

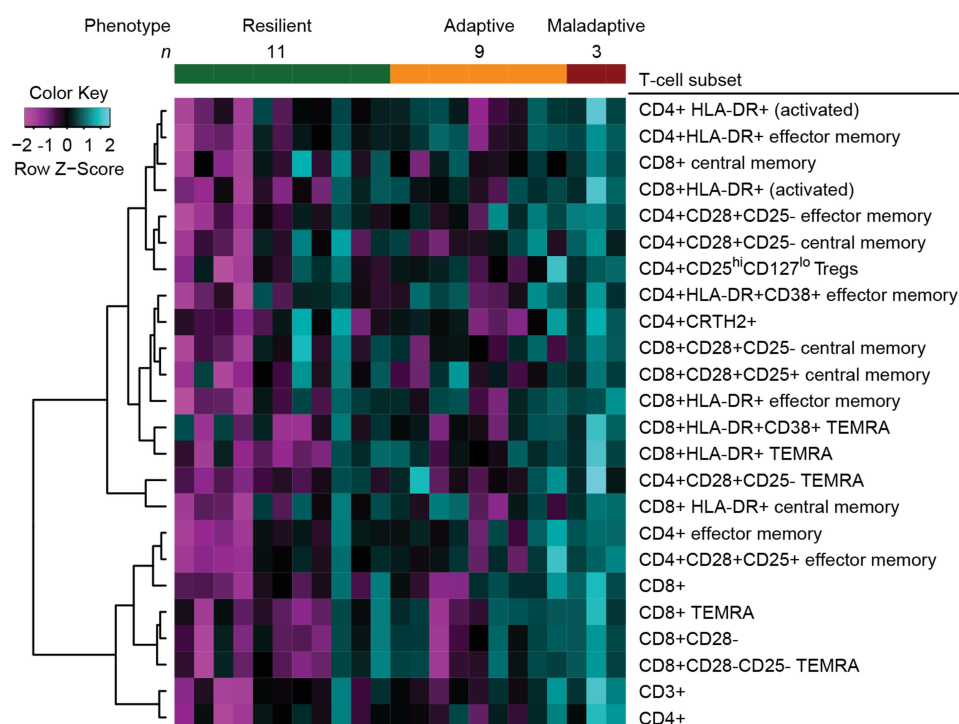


Figure S10. Association of peripheral blood T-cell subsets with HDM-PARC+AA+ evoked phenotypes. Heatmap of T-cell traits that were significantly associated by HDM-PARC+AA+ phenotypes (false discovery rate <0.05). The average value (pre vs. post aeroallergen chamber (ACC) exposure) was used in the heatmap and z-score transformed (mean centered then divided by standard deviation) within each row (cell type). Unsupervised clustering was performed on the row (cell type) using Ward's D linkage and Euclidean distances. Supervised clustering was performed on the column (participants) by phenotype.

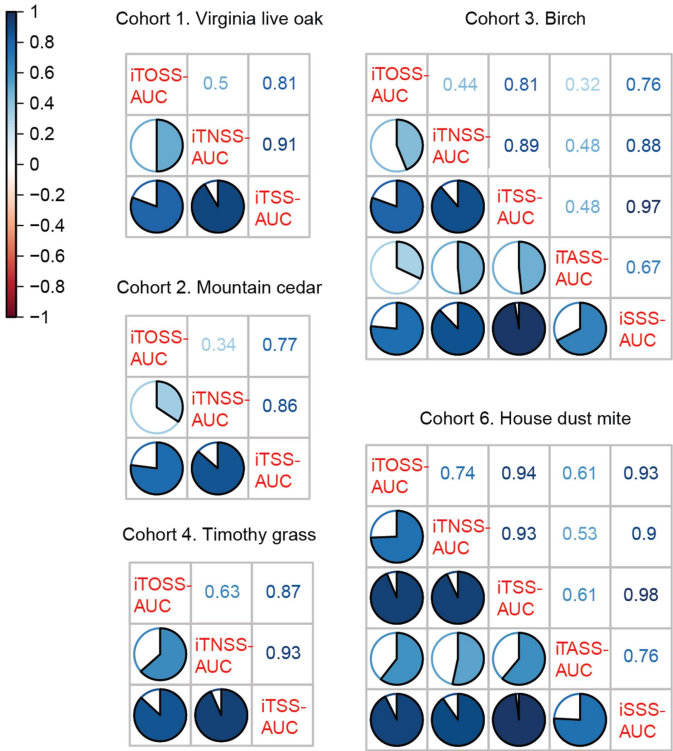


Figure S11. Correlation of symptom severity metrics. Pearson's correlation coefficient and pie chart illustrating Pearson's correlation coefficient to demonstrate the associations of disease severity metrics in the indicated cohorts. *P* determined by Fisher's z-transformation.

752 **Supplementary Tables**

753 Supplementary **Tables S1-S12** are provided here below. **Table S8** and **Table S9** are
754 provided together in a separate excel sheet.

755

Table S1. Cohorts studied and challenges.										evaluated in current study			
Cohort [†]	Date of study	Disease	Aero-allergen	n	# Exp.	Hrs./Exp.	sAEs	Disease severity metric ⁺	Delivered antigen concentration	Symptom severity	Blood traits	Nasal gene expression	Ref.
1	July. 2012	ARC	VLO	34	2	3	0	iTSS	10,000 ± 2,000 grains/m ³	X	-	-	(¹)
2	July. 2011	ARC	MC	21	2	3	0	iTSS	12,500 ± 2,500 grains/m ³	X	-	-	(¹)
3	Nov. 2020	ARC or ARC+AA	Birch	24 [*]	2	3	0	iSSS	3,500 ± 750 grains/m ³	X	-	-	<i>under review</i>
4	Jul. 2013	ARC	Timothy grass	22	2	3	0	iTSS	4,000 ± 1,000 grains/m ³	X	-	-	(²)
5	Jul. 2013	ARC	MC	92	4	1x3 hrs, 3x8hrs	0	iTNSS	12,500 ± 2,500 grains/m ³	X	-	-	not published (part of a clinical study)
6	Dec 2019	ARC+AA	HDM	24	1	5	0	iSSS, rSSS, FEV1, ACQ-7	240.51 ng/m ³	X	X	X	current study

[†], As described, the objective of our study was to test whether the paradigm of evoked phenotypes extends beyond persons with HDM-associated PARC without asthma, as we previously reported¹¹. To do so, we undertook a comprehensive evaluation of challenge studies conducted in persons with (i) ARC attributable to seasonal aeroallergens [cohorts 1-5], and (ii) HDM-associated PARC with AA [cohort 6]. These six challenge cohorts allowed us to scale duration (3 to 8 hours) and frequency (1 to 4) of exposures, diversity of aeroallergens (seasonal and perennial), and both elements of the two-compartments/one-disease paradigm (ARC and AA).

^{*}, 8 of the 24 cohort 3 participants had physician-diagnosed asthma (i.e., ARC+AA)

⁺, "i" indicates instantaneous scores, "r" indicates reflective scores

AA, allergic asthma; ACQ-7, asthma control questionnaire-7; ARC, allergic rhinoconjunctivitis; FEV1, forced expiratory volume in 1 second; HDM, house dust mite; hr, hour; MC, mountain cedar; SSS, summated symptom score; TNSS, total nasal symptom score; TSS, total symptom score; VLO, Virginia live oak

Table S2. Detailed descriptions and statistical methods for each panel shown in the main text Figures 1-7 and supplementary Figures S1-S11.

Figure	Statistics/Analyses
Fig. 1A-D	No statistical analysis was performed.
Fig. 2A-C	<i>Top to bottom;</i> Data from participants exposed to VLO (cohort 1; n=34), mountain cedar (cohort 2; n=23), and Timothy grass (cohort 4; n=22). <i>Top left:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 14 iTSS measurements recorded in the ACC identified 3 clusters which were termed evoked phenotypes [Resilient (R), Adaptive (A), Maladaptive (M)] (A-B) or 2 clusters which were termed evoked phenotypes [Resilient (R), Maladaptive (M)] (C) based on symptom severity pattern trajectories. No statistical analysis was performed. <i>Bottom left:</i> Mean (SEM) iTSS or at 30-minute intervals in the persons who completed the ACC challenge study by evoked phenotypes. <i>Top right:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 14 iTSS measurements recorded in the ACC showing a <i>post-hoc</i> analysis identifying sub-groups within R. <i>Bottom right:</i> Mean (SEM) iTSS or at 30-minute intervals in the R persons who completed the ACC challenge study by sub-groups.
Fig. 2D	<i>Top to bottom;</i> Data from participants exposed to Birch (cohort 3; n=24). <i>Top:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 14 iSSS measurements recorded in the ACC identified 3 clusters which were termed evoked phenotypes [Resilient (R), Adaptive (A), Maladaptive (M)]. No statistical analysis was performed. <i>Bottom:</i> Mean (SEM) iTSS or at 30-minute intervals in the persons who completed the ACC challenge study by evoked phenotypes.
Fig. 2E	<i>Top Left:</i> Schema of cohort 5 study design. <i>Top Right:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 7 iTNSS measurements recorded

	in the ACC identified 3 clusters which were termed evoked phenotypes [Resilient (R), Adaptive (A), Maladaptive (M)] based on symptom severity pattern trajectories. No statistical analysis was performed. <i>Bottom:</i> Mean (SEM) iTNSS at 30-minute intervals in the participants (n=92) who completed the ACC challenge study by R, A, M phenotypes.
Fig. 2F	Mean (SEM) iTNSS-AUC by evoked phenotype across each study day. <i>P</i> determined using the likelihood ratio test to compare the indicated phenotypes on study day 1 and study day 4.
Fig. 2G	Meta-analysis from the combination of data from participants exposed to VLO (cohort 1; n=34), mountain cedar (cohort 2; n=23), and Timothy grass (cohort 4; n=22). <i>Top:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 14 iTSS measurements recorded in the ACC identified 5 clusters which were termed evoked phenotypes based on symptom severity pattern trajectories. No statistical analysis was performed. <i>Bottom:</i> Mean (SEM) iTSS or at 30-minute intervals in the persons who completed the ACC challenge study by evoked phenotypes.
Fig. 2H	Meta-analysis from the combination of data from participants exposed to VLO (cohort 1; n=34), mountain cedar (cohort 2; n=23), and Timothy grass (cohort 4; n=22). <i>Top:</i> Box plot showing adjusted number of positive SPTs by evoked phenotypes from the meta-analysis. I negative binomial GLM with number of positive SPTs as the outcome and evoked phenotypes (categorical) and cohort (categorical) as the predictors. As the link for the model was log, the number of positive SPTs was divided the exponentiated coefficient for the corresponding cohort to calculate a number of positive SPTs adjusted by cohort, and that value was plotted by evoked phenotype. <i>P</i> value was determined using a LRT (adjusted by cohort). <i>Bottom:</i> Bar plots depicting $\text{slgE}^{\text{lo/hi}}$ for each evoked phenotype. <i>P</i> value was determined by a logistic regression model with $\text{slgE}^{\text{lo/hi}}$ as the outcome and evoked phenotypes (categorical) and cohort (categorical) as the predictors with a LRT (adjusted by cohort).
Fig. 2I	Bar plot showing the proportion of patients of the evoked phenotypes (outcome) by cohorts (predictor). <i>P</i> values determined by Fisher's exact

	test
Fig. 2J	Boxplots of the mean exposure 1 and 2 iTSS-AUC by cohorts. <i>P</i> values determined by LRT
Fig. 3A	<i>Left:</i> Mean (SEM) iSSS at 30-minute intervals in the 23 HDM-PARC+AA+ persons who completed the ACC challenge study. <i>Right:</i> Unsupervised hierarchical clustering with Manhattan distances and Ward's D linkage of all 11 iSSS measurements recorded in the ACC identified 3 clusters which were termed HDM-PARC+AA+ evoked phenotypes [Resilient (R), Adaptive (A), Maladaptive (M)] based on symptom severity pattern trajectories. No statistical analysis was performed.
Fig. 3B	<i>Top:</i> Schema of study design. <i>Middle (symptoms):</i> Line plots: Mean (SEM) of Run-In rSSS (recorded twice daily AM/PM on study days -4 to -1), iSSS (recorded at 30-minute intervals during the ACC), and natural setting rSSS (recorded twice daily AM/PM on study days 1 to 4) by HDM-PARC+AA+ phenotypes defined in the ACC. No statistical analysis was performed. Box plots: Run-in mean rSSS (average rSSS recordings from study days -4 to -1), iSSS-AUC (computed using all 11 iSSS recordings in the ACC), and Natural setting mean rSSS (average rSSS recordings from study days 1 to 4) by HDM-PARC+AA+ phenotypes. <i>P</i> values determined by likelihood ratio test (LRT). <i>Bottom (FEV1):</i> Line plots: Mean (SEM) of FEV1 recorded twice daily AM/PM on study days -4 to -1, FEV1 recorded at 30-minute intervals during the ACC, and FEV1 recorded twice daily AM/PM on study days 1 to 4 by HDM-PARC+AA+ phenotypes defined in the ACC. No statistical analysis was performed. Box plots: Run-in mean FEV1 (average FEV1 recordings from study days -4 to -1), FEV1-AUC (computed using all 11 FEV1 recordings in the ACC), and Natural setting mean FEV1 (average FEV1 recordings from study days 1 to 4) by HDM-PARC+AA+ phenotypes. <i>P</i> values determined by LRT

Fig. 3C	Bar plot showing the proportion of patients treat for drop in FEV1 (supplementary methods) by evoked phenotypes. <i>P</i> values determined by Fisher's exact test
Fig. 4A	<i>Top</i> : Mean (SEM) of iTSS (top) and iTASS (bottom) recorded at 30-minute intervals in the ACC by HDM-PARC+AA+ phenotypes. No statistical analysis was performed. <i>Bottom</i> : Boxplots of iTSS-AUC (top) and iTASS-AUC (bottom) by HDM-PARC+AA+ phenotypes. <i>P</i> values determined by LRT
Fig. 4B	Schema. No statistical analysis was performed.
Fig. 4C	The proportion of persons identified as having the resilient (R), adaptive (A), or maladaptive (M) evoked phenotypes (outcome) by Run-In rSSS tertiles (1 st : lowest rSSS, 2 nd : intermediate rSSS, 3 rd : highest rSSS; tertiles were computed using average-rSSS recordings during study days -4 to -1) (predictor). <i>P</i> values determined by Fisher's exact test.
Fig. 4D	<i>Top</i> : Mean (SEM) of iSSS (top) and FEV1 (bottom) recorded at 30-minute intervals in the ACC by Run-In rSSS tertiles (1 st , 2 nd , and 3 rd). No statistical analysis was performed. <i>Bottom</i> : iSSS-AUC (top) and FEV1-AUC (bottom) by Run-In rSSS tertiles (1 st , 2 nd , and 3 rd). <i>P</i> values determined by LRT
Fig. 4E	Box plots of (left to right) iSSS-AUC, iTASS-AUC, iTSS-AUC, and FEV1 (L)-AUC by evoke phenotypes and Run-In rSSS tertiles. <i>P</i> values are comparing R to 1 st tertile, A to 2 nd tertial, M to 3 rd tertile, and A to 3 rd tertile were determined using a linear GEE model with the correspond AUC as the outcome and group membership (evoked phenotype vs. tertile) as the explanatory variable with an exchangeable correlation structure.
Fig. 4F	Box plots ACQ-7 measured at visit 2 by (left to right) evoked phenotypes, run-in rSSS tertiles, CD8-CD4 ^{hi/lo} strata, Eos ^{hi/lo} strata, Neut ^{hi/lo} strata, and sIgE ^{hi/lo} strata. Cutoffs used for the peripheral blood trait strata are detailed in the supplementary methods. <i>P</i> values determined using Kruskal-Wallis test.
Fig. 4G	<i>Cohort A</i> : A group of 23 HDM-PARC+AA- persons that were challenged with HDMs for 4 consecutive 3-hour exposures in the ACC (Ref. ¹¹). The per exposure iTSS-AUC was calculated as described in section C.3 and

	were then averaged together. For this cohort from our previous study¹¹, the 7 components of iTSS were scored on a Likert score range from 0 to 4, with the iTSS ranging from 0 to 28. Thus, to perform comparisons between this HDM-PARC+AA- cohort and our current HDM-PARC+AA+ cohort 6 who scores symptoms on a 0 to 4 Likert scale (Table S4), the iTSS-AUC in the cohort of HDM-PARC+AA- persons was multiplied by $\frac{3}{4}$ to normalize to the same scale as the HDM-PARC+AA+ cohort 6 participants. <i>Left:</i> The proportion of persons identified as having the resilient (R), adaptive (A), or maladaptive (M) evoked phenotypes (outcome) by cohort. <i>P</i> values determined by Fisher's exact test. <i>Right:</i> Box plots of iTSS-AUC (outcome) overall and within evoked phenotypes (R, A, and M) by cohort. <i>P</i> values determined by linear model with likelihood ratio test.
Fig. 5A	<i>Left:</i> CD8-CD4 balance clusters identified by unsupervised clustering using baseline (t=0 hours, pre-ACC) peripheral blood CD8+ T-cell counts and CD4:CD8 T-cell ratio values (obtained by flow cytometry) using Euclidean distances and Ward's D linkage. No statistical analysis was performed. <i>Right:</i> Baseline CD8+ and CD4+ T-cell counts and CD4:CD8 T-cell ratio values stratified by the identified CD8-CD4 balance clusters I (designated as CD8^{lo}-CD4^{lo}) and II (designated as CD8^{hi}-CD4^{hi}). Y-axes depicted as exponentiation of log₂ scale. <i>P</i> values determined by linear model with LRT using log₂-transformed cell counts.
Fig. 5B	The proportion of persons with the indicated peripheral blood trait strata (outcome) by HDM-PARC+AA+ phenotypes (predictor). <i>P</i> values determined by Fisher's exact test. Cutoffs used for the peripheral blood trait strata are detailed in the supplementary methods.
Fig. 5C	Mean (SEM) of iSSS recorded at 30-minute intervals in the ACC in HDM-PARC+AA+ persons stratified by the indicated peripheral blood trait strata. <i>P</i> values determined by linear generalized estimating equations (GEE) model with an autoregressive 1 correlation structure and adjusted by time (in minutes).
Fig. 5D-E	Run-in mean rSSS (recorded twice daily AM/PM on study days -4 to -1), iSSS-AUC (computed using all 11 iSSS recordings in the ACC), and the natural setting mean rSSS (recorded twice daily AM/PM on study days 1 to 4) in HDM-PARC+AA+ persons stratified by (D) CD8-CD4, or (E) eosinophil (Eos) strata. <i>P</i> values determined by linear model with LRT.

Fig. 5F	Coefficient of determination (r^2) determined using linear regression with iSSS-AUC as the dependent variable and peripheral blood trait strata (CD8-CD4 ^{hi/lo} , Eos ^{hi/lo} , Neut ^{hi/lo} , or slgE ^{hi/lo}) as the independent variable. <i>P</i> values determined by LRT.
Fig. 6A	Trajectories of levels of the indicated T-cell subsets ($\log_2$ transformed) from baseline (t=0 hours, pre-ACC) to post ACC (t=5 hours after initiating HDM dispersal in the ACC) in HDM-PARC+AA+ persons stratified by (top to bottom) evoked phenotypes, CD8-CD4 ^{hi/lo} , Eos ^{hi/lo} , slgE ^{hi/lo} , and run-in rSSS tertiles. <i>P</i> values determined by linear GEE model with an exchangeable correlation structure and adjusted by time as a factorial variable with ANOVA.
Fig. 7A	Schema. No statistical analysis was performed.
Fig. 7B	For analysis of single genes (<i>FLG</i>, <i>OSM</i>, <i>POSTN</i>), non-transformed, normalized gene counts were subjected to a GEE-GLM model based on the Gamma distribution with a log link function. Models were adjusted by time (as a categorical variable; t=0 vs. t=5 hours in the ACC) and an exchangeable correlation structure. DESeq models the gene counts with a negative binomial distribution, assumes the shot noise term (the size factor) is modeled by Poisson distribution, and normalizes the gene counts by dividing them by the size factor (Anders S, and Huber W., 2010). Since the negative binomial distribution can arise from a Gamma-Poisson mixture, the normalized gene counts would follow a Gamma distribution. <i>Ref: Anders S, and Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11(10):R106.</i> Columns 1-3: Trajectories (during the ACC) of $\log_2$-normalized gene expression (NGE) of (<i>left to right</i>) <i>FLG</i>, <i>POSTN</i>, and <i>OSM</i> in HDM-PARC+AA+ nasal cell transcriptomes stratified by Eos, slgE, and CD8-CD4 peripheral blood strait strata, evoked phenotypes, and Run-in rSSS tertiles. Column 4: To generate the z-score of the asthma^{up} gene signature, the $\log_2$-normalized expression of each gene within the signature was z-transformed (mean centered then divided by standard deviation) across all samples and then averaged for all gene genes in the signature. Depicted are the trajectories (during the ACC) of z-scores of the asthma^{up} gene signature in HDM-PARC+AA+ nasal cell transcriptomes stratified by Eos, slgE, and CD8-CD4 peripheral blood strait strata,

	evoked phenotypes, and Run-in rSSS tertiles. Statistical methods for single gene analysis longitudinally (during ACC) included a GEE GLM based on the Gamma distribution and adjusted by time with an autoregressive(1) correlation structure, with an ANOVA. Statistical methods for z-scores analysis longitudinally (during ACC) included a GEE GLM based on the normal distribution and adjusted by time with an autoregressive(1) correlation structure with an ANOVA.
Fig. 7C	Schema. No statistical analysis was performed.
Fig. S1A-C	Schemas of the (A-B) cohort 6 study design and (C) disease severity metrics. No statistical analysis was performed.
Fig. S2	Data derived from Cohort 5. Mean (SEM) iTNSS at 30-minute intervals in participants during Exposures 2, 3, and 4 by evoked phenotypes [resilient (R) and maladaptive (M)].
Fig. S3	Pearson's correlation coefficient and pie chart of the area-under-the curve (AUC) metrics of FEV1, iTNSS, iTOSS, and iTASS was performed using Pearson's correlation coefficient. <i>P</i> values were determined by Fisher's z-transformation.
Fig. S4A	<i>Top</i> (rows 1-2) (Run-in, pre-ACC) Left: Mean (SEM) of rTSS and rTASS recorded twice daily AM/PM on study days -4 to -1 by HDM-PARC+AA+ evoked phenotypes. No statistical analysis was performed. <i>Right</i>: Run-in mean rTSS and rTASS (average recordings from study days -4 to -1) by HDM-PARC+AA+ evoked phenotypes. <i>P</i> values determined by LRT. <i>Middle</i> (rows 3-4) (ACC) Left: Mean (SEM) of iTSS and iTASS recorded at 30-minute intervals in the ACC by HDM-PARC+AA+ evoked phenotypes. No statistical analysis was performed. <i>Right</i>: iTSS-AUC and iTASS-AUC (computed using all 11 iTSS or iTASS recordings in the ACC, respectively) by HDM-PARC+AA+ evoked phenotypes. <i>P</i> values determined by LRT <i>Bottom</i> (rows 5-6) (post-ACC)

	Left: Mean (SEM) of rTSS and rTASS recorded twice daily AM/PM on study days 1 to 4 by HDM-PARC+AA+ evoked phenotypes. No statistical analysis was performed. <i>Right:</i> Mean rTSS and rTASS (average recordings from study days 1 to 4) by HDM-PARC+AA+ evoked phenotypes. <i>P</i> values determined by LRT.
Fig. S4B-C	Same as Figure S4A [<i>middle</i> ; ACC (HDM 5-hours)] except stratification and analysis shown here is by Run-In rSSS tertiles.
Fig. S5	Same as Figure 4E except for ACQ-7 scores obtained at visits 4 (top) and 5 (bottom).
Fig. S6A-B	Same as Figure 5C-E, except for iTSS, iTASS, iSSS, and FEV1 metrics each as an area-under-the-curve (AUC) and trajectories (during the ACC) stratified by peripheral blood trait strata (A) CD8 ^{hi/lo} and (B) Eos ^{hi/lo} .
Fig S7	The proportion of persons with the indicated peripheral blood trait strata (outcome) by Run-In rSSS tertiles (1 st , 2 nd , and 3 rd ; predictor). <i>P</i> values determined by Fisher's exact test. Cutoffs used for the peripheral blood trait strata are detailed in the supplementary methods.
Fig. S8A-B	Same as Figure 5C-E, except for iTSS, iTASS, iSSS, and FEV1 metrics each as an area-under-the-curve (AUC) and trajectories (during the ACC) stratified by peripheral blood trait strata (A) Neut ^{hi/lo} and (B) slgE ^{hi/lo} .
Fig. S9A-B	Same as Figure 5D-E, except stratified by (A) Neut ^{hi/lo} and (B) slgE ^{hi/lo} strata.
Fig. S10	Heatmap of T-cell traits that significantly (false discovery rate <0.05) associated with the HDM-PARC+AA+ evoked phenotypes. The average value (pre [t=0 hr.] vs. post [t=5 hours] aeroallergen chamber (ACC) exposure) was used in the heatmap and z-score transformed (mean centered then divided by standard deviation) within each row (cell type). Unsupervised clustering was performed on the row (cell type) using Ward's D linkage and Euclidean distances. Supervised clustering was performed on the column (participants) by evoked phenotypes.
Fig. S11	Pearson's correlation coefficient and pie chart of the area-under-the curve (AUC) metrics of iTNSS, iTOSS, iTASS, and iSSS was performed using Pearson's correlation coefficient. <i>P</i> values were determined by Fisher's z-transformation.

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Table S3. Inclusion/Exclusion Criteria applied to Cohort 6 HDM-PARC+AA+ participants**Inclusion Criteria**

1. Subject demonstrates understanding of the study and has provided an appropriately signed and dated informed consent form.
2. Male or Female, 18 to 65 years of age.
3. Persons with mild-to-moderate intermittent to persistent allergic asthma must have a history of hypersensitivity to HDMs for a minimum of 2 years prior to the Screening visit.
4. Positive response by prick skin test to *D. pteronyssinus* of ≥ 5 mm.
5. Subjects demonstrates 12% or greater improvement in FEV₁ and a post-bronchodilator FEV₁/FVC (forced vital capacity) ratio of $\geq 70\%$.
6. Subject understands and is willing, able and likely to comply with study procedures and restrictions.
7. Females must have a negative urine pregnancy test at Visit 1 and Visit 2 prior to chamber entry.
8. All women of childbearing potential must agree to use a medically acceptable form of birth control throughout the study duration and for at least 1 month prior to Visit 1.

Subject must be able to read, comprehend, and record all information in English.

Exclusion Criteria

1. Currently involved in another study.
2. Female subject who is pregnant or lactating.
3. Any kidney, liver, heart, brain/nervous system, blood or stomach problems or other medical illness that the study physician believes will interfere with the study.
4. Any abnormalities found during physical exam that the study physician believes will interfere with the study.
5. Respiratory and/or bacterial sinusitis infection during the past fourteen (14) days prior to the Screening visit.
6. Participated in a trial with an investigational drug in the past thirty (30) days prior to the Screening visit.
7. Current alcohol or drug abuse or history of the same in the past three (3) years.
8. Use of disallowed medications (**Table S11**).
9. On immunotherapy and/or increasing doses of immunotherapy.
10. History of asthma requiring hospitalization, emergency room visit, or use of chronic systemic corticosteroids.
11. Unable to withhold specified asthma medications for specified times prior to chamber exposure.
12. FEV₁/FVC <70% of predicted

HDM, house dust mite; FEV, forced expiratory volume, FVC, forced vital capacity.

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Table S4. Symptom scoring systems.

Table S4a. Total Symptom Score (TSS) scoring system used in Cohorts 1-6*

Symptom	Score range
TNSS	0-12
Rhinorrhea ¹	0-3
Congestion ¹	0-3
Sneezing ¹	0-3
Nasal itching ¹	0-3
TOSS	0-9
Ocular redness ²	0-3
Tearing ²	0-3
Ocular itching ²	0-3
Total symptom score (TSS) = TNSS + TOSS	0-21

*Cohort 5 was evaluated using the TNSS scoring component

^{1,2}Scored on a Likert-scale of 0=absent, 1=mild, 2=moderate, 3=severe
Component of ¹TNSS, ²TOSS

Table S4b. Summated Symptom Score (SSS) scoring system used in Cohorts 3 and 6.

Symptom	Score range
TNSS	0-12
Rhinorrhea ¹	0-3
Congestion ¹	0-3
Sneezing ¹	0-3
Nasal itching ¹	0-3
TOSS	0-9
Ocular redness ²	0-3
Tearing ²	0-3
Ocular itching ²	0-3
Total symptom score (TSS) = TNSS + TOSS	0-21
TASS	0-9
Cough ³	0-3
Wheeze ³	0-3
Dyspnea ³	0-3
Summated symptom score (SSS) = TSS + TASS	0-30

^{1,2,3}Scored on a Likert-scale of 0=absent, 1=mild, 2=moderate, 3=severe
Component of ¹TNSS, ²TOSS, ³TASS

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Table S5. Antibody panels used for flow cytometry in whole blood.		
Panel	Antibodies (clones)	Manufacturer
T cells	CD3 BUV395 (UCHT1), CD4 APC-R700 (RPA-T4), CD8 V500 (SK1), CD25 PE (M-A251), CD28 BUV737 (CD28.2), CD38 BV421 (HIT2), CD39 APC (TU66), CD45RA FITC (HI100), CD69 BV650 (FN50), CD95 PE-CF594 (DX2), CD127 Pe-Cy7 (HIL-7R-M21), CCR7 PerCP-Cy5.5 (150503).	BD Biosciences
	CD294 BV711 (BM16), HLA-DR APC-Cy7 (L243)	Biolegend
B cells	CD5 PE (UCHT2), CD10 PE-CF594 (HI10 α), CD19 APC-Cy7 (SJ25C1), CD20 BUV395 (2H7), CD21 BV210 (1048), CD27 APC-R700 (M-T271), CD95 BV421 (DX2), IgM BV711 (G20-127), IgG BB515 (G18-145), I	BD Biosciences
	CD38 Pe-Cy7 (HB7), IgD BV785 (IA6-2).	Biolegend
	IgA APC (IS11-8E10)	Miltenyi Biotec
Innate	CD3 BUV395 (UCHT1), CD8 V500 (SK1), CD11c Pe-Cy5 (B-Ly6), CD14 BB700 (M ϕ P9), CD16 FITC (3G8), CD19 APC-R700 (HIB19), CD56 BV650 (NCAM16.2), CD123 Pe-Cy7 (7G3), CD141 BV786 (1A4), CCR3 PE-CF594 (5E8), Siglec8 BV421 (837535), HLA-DR BUV737 (G46-6).	BD Biosciences
	MAIT/TCR Va7.2 APC (3C10), iNKT/TCR Va24-Ja18 PE (6B11)	Biolegend

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Table S6. Cohort 6 characteristics and demographics.

Variable		Overall	HDM-PARC+AA+ phenotype			<i>P</i>
			Resilient	Adaptive	Maladaptive	
n		23	11	9	3	
Age		44 (36-51)	36 (35-47)	45 (35-51)	53 (46-55)	0.287
Height		64.5 (63.25-66.25)	65 (63.75-67.25)	64.5 (63.5-66)	62 (62-63)	0.254
Weight		205 (169.5-227)	214 (176.5-240.5)	200 (187-211)	145 (144-181.5)	0.337
BMI		33.6 (27.2-39)	35.6 (28.6-39.6)	33.6 (30.2-36.6)	26.2 (25.55-33.05)	0.584
Sex	Female	15 (65.22)	6 (54.55)	6 (66.67)	3 (100.00)	0.432
	Male	8 (34.78)	5 (45.45)	3 (33.33)	0 (0.00)	
Race	EA	6 (26.09)	1 (9.09)	4 (44.44)	1 (33.33)	0.430
	HA	13 (56.52)	7 (63.64)	4 (44.44)	2 (66.67)	
	AA	4 (17.39)	3 (27.27)	1 (11.11)	0 (0.00)	
Income	<\$35k	2 (8.70)	2 (18.18)	0 (0.00)	0 (0.00)	0.091
	\$35k-\$75k	12 (52.17)	4 (36.36)	7 (77.78)	1 (33.33)	
	\$75k-\$100k	6 (26.09)	4 (36.36)	0 (0.00)	2 (66.67)	
	≥\$100k	3 (13.04)	1 (9.09)	2 (22.22)	0 (0.00)	
	0%	12 (52.17)	7 (63.64)	2 (22.22)	3 (100.00)	
Time Spent Outdoors	25%-50%	7 (30.43)	2 (18.18)	5 (55.56)	0 (0.00)	0.172
	50%-75%	3 (13.04)	1 (9.09)	2 (22.22)	0 (0.00)	
	75%-100%	1 (4.35)	1 (9.09)	0 (0.00)	0 (0.00)	

EA, European American; HA, Hispanic American; AA, African American; k, x1000

P values determined by Kruskal-Wallis (age, height, weight, BMI) and Fisher's exact test (sex, race, income, time spent outdoors).

Table S7. Skin prick test wheal reactivity in Cohort 6 HDM-PARC+AA+ participants.

Antigen	Skin prick test (SPT) wheal size (mm)				<i>P</i>
	Overall	HDM-PARC+AA+ phenotype			
		Resilient	Adaptive	Maladaptive	
HDM*	7 (6-9)	7 (6-9)	7 (7-8)	7 (6-10)	0.978
Ragweed	5 (3-6)	5 (3-6)	5 (0-6)	5 (4-6)	0.987
Mtn. Cedar	9 (6-13)	6 (5-11)	11 (9-13)	12 (10-15)	0.210
Cockroach	5 (4-5)	5 (2-5)	4 (3-5)	5 (5-6)	0.371
Cat	6 (3-8)	6 (4-8)	5 (3-10)	3 (2-6)	0.709

P, determined by Kruskal-Wallis test

*Positive SPT to HDM of ≥ 5 mm was required for study entry

Mtn, Mountain; SPT, skin prick test.

Table S8. Peripheral blood lymphocyte subsets evaluated in Cohort 6 HDM-PARC+AA+ participants.

This table is provided separately in an excel spreadsheet.

Table S9. Innate immune and B cell subsets evaluated in Cohort 6 HDM-PARC+AA+ participants.

This table is provided separately in an excel spreadsheet.

Table S10. Cohorts 1-5 demographics.						
		Evoked phenotype				
		Overall	Resilient	Adaptive	Maladaptive	<i>P</i>
Cohort 1	<i>n</i>	34	15	11	8	
	Age	44 (39-50)	44 (42-46)	39 (29-52)	48 (42-57)	0.593
	Male	11 (32.4)	4 (26.7)	3 (27.3)	4 (50.0)	0.575
	Ethnicity					0.466
	EA	17 (50.0)	9 (60.0)	4 (36.4)	4 (50.0)	
	AA					
	HA	17 (50.0)	6 (40.0)	7 (63.6)	4 (50.0)	
Cohort 2	<i>n</i>	21	9	9	3	
	Age	44 (32-49)	43 (33-45)	47 (29-51)	49 (48-49)	0.673
	Male	6 (18.8)	1 (11.1)	1 (11.1)	1 (33.3)	0.546
	Ethnicity					0.511
	EA	13 (40.6)	2 (22.2)	3 (33.3)	2 (66.7)	
	AA					
	HA	17 (53.1)	7 (77.8)	5 (55.6)	1 (33.3)	
Cohort 3	<i>n</i>	24	3	7	14	
	Age	40 (38-49)	40 (34-46)	39 (37-50)	42 (39-47)	0.901
	Male	12 (50.0)	2 (66.7)	3 (42.9)	7 (50.0)	>0.999
	Ethnicity					0.805
	EA	9 (37.5)	1 (33.3)	3 (42.9)	5 (35.7)	
	AA	3 (12.5)	1 (33.3)	1 (14.3)	1 (7.1)	
	HA	12 (50.0)	1 (33.3)	3 (42.9)	8 (57.1)	
Cohort 4*	Asthma [#]	8 (33.3)	2 (66.7)	2 (28.6)	4 (28.6)	0.590
	<i>n</i>	22	15	0	7	
	Age	44 (35-48)	45 (38-48)		38 (29-52)	0.621
	Male	12 (54.5)	8 (53.3)		4 (57.1)	>0.999
	Ethnicity					>0.999
	EA	11 (50.0)	7 (46.7)		4 (57.1)	
	AA	1 (4.5)	1 (6.7)		0 (0.0)	
Cohort 5	HA	9 (40.9)	6 (40.0)		3 (42.9)	
	Other	1 (4.5)	1 (6.7)		0 (0.0)	
	<i>n</i>	92	27	47	18	
	Age	43 (34-49)	44 (32-48)	42 (35-50)	43 (38-47)	0.959
Cohort 5	Male	24 (26.1)	8 (29.6)	12 (25.5)	4 (22.2)	0.859
	Ethnicity					0.195

Table S11. Restricted medications for Cohort 6 HDM-PARC+AA+ study.	
Medication	Required withholding interval prior to Visit 2 (ACC)
Topical/Oral/Nasal Decongestants	10 days
Ocular allergy preparations	10 days
Short-acting antihistamines (nasal and ocular)	5 days
Long-acting antihistamines (nasal and ocular)	10 days
Over-the-counter cough and cold preparations or sleep agents	10 days
Airozan® (OTC food supplement /diet leukotrienes)	7 days
Cromolyn, nedocromil, or Iodoxamine (intranasal, ocular, or oral)	14 days
Leukotriene or 5-LO inhibitors	14 days
Inhaled/Oral/Intranasal anticholinergics	14 days
Tricyclic antidepressants	14 days
Monoamine oxidase inhibitors	14 days
Short-acting Beta-agonists	12 hours
Inhaled/intranasal corticosteroids	12 hours
Long-acting Beta-agonists	12 hours
Azoles, anti-fungals	30 days
Immunosuppressive drugs	60 days
The following medications were permitted with the following restrictions: • Aspirin (325 mg/day or less) given continuously as a prophylaxis and therapy initiated prior to Screening (Visit 1) is permitted. No dosage adjustments will be allowed during the study. • Hydrocortisone less than or equal to 1% concentration, or equivalent, covering less than or equal to 20% of the total body surface without occlusion, is permitted. • Other drugs to treat concurrent diseases are allowed; however, dosage and frequency should be kept as constant as possible throughout the study. • Asthma medications must be withheld for 12 hours prior to the start of HDM exposure in the ACC.	

Table S12. Levels of Der p 1 collected in the ACC during the 5-hour HDM exposures in Cohort 6 HDM-PARC+AA+ participants.

Hour of HDM exposure	Cohort 6 HDM-PARC+AA+ group	
	1	2
	Mean Der p 1 (ng/m ³)	
0 (baseline; t=0)	0.00	0.00
1	262.24	154.47
2	261.49	151.96
3	328.64	154.00
4	99.69	285.35
5	293.13	414.05
Average Der p 1 (ng/m³):	249.04	231.97

Shown are the mean Der p 1 levels (ng/m³) assayed by ELISA from samples collected from 3 stations at the indicated time points post exposure. Group 1 and 2 refers to the HDM-PARC+AA+ participants who were challenged with HDM 1 week apart.

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