Baseline Predictors of Symptom Severity
Following Exposure to House Dust Mite in an Antigen Challenge Chamber (ACC)

Abstract #765

D. Ramirez[1], R. Jacobs[1], C. Andrews[2,3], C. Rather[1], N. Harper[2,3], A. Carrillo[2,3], W. He[2,3], and S. K. Ahuja[2,3]

[1]Biogenics Research Chamber, San Antonio, TX. [2]Veterans Administration Center for Personalized Medicine, South Texas Veterans Health Care System, San Antonio, TX.
[3]Department of Medicine, University of Texas Health Science Center, San Antonio, TX.

Introduction

Allergy to house dust mites (HDM) is the major cause of indoor perennial allergic disorders, including asthma, rhinitis, and atopic dermatitis. Treatment modalities have had mixed success and have included efforts to avoid or control mites in the environment, the use of a wide range of systemic and end-organ medications, and specific immunotherapy with both injection and oral methods with HDM preparations. The symptoms from HDM exposure tend to be perennial with acute exacerbations at times of increased house dust exposure. However, 15% of individuals will demonstrate positive reactivity to a prick test (SPT) to HDM yet have no symptoms with exposure [1]. These symptoms and their pattern often mimic those seen with chronic idiopathic rhinitis, often aggravated by exposure to other environmental co-factors such as weather changes, smoke and other respiratory irritants. These patients cannot be recognized in the natural setting and, if included in a natural setting study, failure is a likely possibility. Another source of confounding in clinical trials could the variable exposure to HDM in the natural setting, invoking the need for more rigorous clinical trials[10]. One way to mitigate confounding factors is by the use of an Allergen Challenge Chamber (ACC).

Recent studies have demonstrated that exposure to aeroallergens in an Allergen Challenge Chamber (ACC) is a sensitive, specific and reproducible method for elicitation of AR symptoms [2, 3]. A recent NIH workshop recognized the ACC model as a potential tool for proving the efficacy of novel products for allergen immunotherapy [4]. Most of these studies, including ours, have focused on pollen exposures [4, 5]. We sought to investigate HDM related issues in the Biogenics Allergen Chamber to minimize other environmental co-factors and attempt to find factors predictive of a response within the ACC.

Objectives

In a concurrent study (Jacobs et al, Poster# 765), we established the conditions in our ACC (Biogenics Research Chamber) necessary for the elicitation of symptoms in HDM+ individuals. In the present study, we investigated whether SPT reactivity to pollens and German cockroach stratifies the AR-related responses in HDM+ individuals both in the natural setting and following induction of symptoms after exposure to HDM in our ACC.

Methods

- Participants of both genders, 18 to 70 years were enrolled, and classified as HDM+ or HDM- according to a positive versus negative response, respectively, to skin prick test (SPT) to D. pteronyssinus (≥ 5 mm).
- The study comprised 4 consecutive phases (Figure 1A): (i) a run-in phase in the natural setting comprised 4 days prior to first of series of HDM challenges in the ACC (ACC-I); (ii) ACC-I with exposure of 3 hours on each of four consecutive days (exposures 1 to 4) to a minced, purified mite body preparation of Dermatophagoides pteronyssinus; (iii) Observation phase in the natural setting of 38 days prior to ACC-II; and (iv) ACC-II with exposure to HDM using identical conditions as those employed for ACC-I. Twenty-one HDM+ and 15 HDM- participants completed all 4 study phases.
- Instantaneous total symptom scores (iTSS, scale 0-28) were measured at baseline and 30 minute intervals during ACC-I and ACC-II. Reflective (rTSS) was also recorded in the morning and evening during the run-in and observation phases. Nasal symptoms comprised of nasal congestion, nasal itching, sneezing, and runny nose. Ocular symptoms were of itching, tearing, and redness. Nasal and ocular symptoms were recorded on a Likert scale of (absent symptoms) to 4 (very severe symptoms).
- The level of T-cell activation markers, CD4+CD69+HLA-DR+ and CD8+CD69+HLA-DR+ as well as the CD4:CD8 T-cell ratio, a marker of immune activation in some conditions, were accessed by flow cytometry using standard methods. The antibodies for flow cytometry were CD3 V500, CD4 PE-Cy7, CD69 PE, and HLA-DR APC (BD Biosciences).

Study Results

Table 1. Skin test responses

Allergen	HDM+ (n=21)
Mountain Cedar	14 (67%)
Virginia Live Oak	7 (33%)
Fall (Cedar) Elm	2 (10%)
Pecan Pollen	3 (14%)
Arizona Ash	0
Ragweed, Short	0
Ragweed, Mixed	0
Spiny Pigweed	0
Bermuda Grass	0
Timothy Grass	0
Mold Mix I	0
Mold Mix II	0
Dog Epithelium	0
Cat Hair	3 (14%)
D. pteronyssinus	21 (100%)
D. farinae	18 (86%)
German Cockroach	9 (43%)

Table 2. Characteristics of study participants

Age, Mean (SD)	41.1 (8.7)
Gender, # Male (%)	6 (28.6)
Ethnicity, # (%)	
Caucasian	7 (33.3)
Hispanic	12 (57.1)
African American	2 (9.5)
IgE specific to HDM Der p1, ng/ML	0.6 (0-2.4)
Peripheral eosinophils, %	1.9 (1.1-2.4)
Positive SPT of 17 aeroallergens tested*	3 (3-5)
Positive SPT to any pollen, n (%)	14 (66.7)
Positive SPT to German cockroach, n (%)	9 (42.9)
Positive SPT to Mt. Cedar SPT, n (%)	14 (66.7)

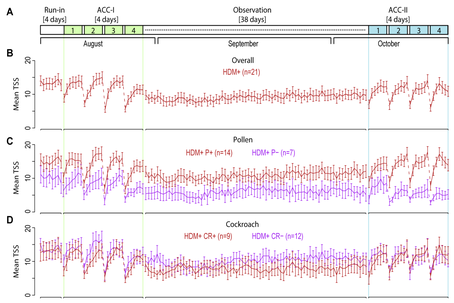
*Median (interquartile range, IQR). SPT, skin prick test. SD, standard deviation

Table 3. Association of demographic, laboratory and skin test reactivity characteristics with TSS

	maximum TSS								DTSS			
	Run-in		ACC-I		Observation		ACC-II		ACC-I		ACC-II	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Gender	0.36	0.108	0.34	0.137	0.20	0.396	0.25	0.273	0.26	0.259	0.07	0.757
Age	0.29	0.201	0.05	0.841	0.12	0.598	0.10	0.651	0.05	0.825	-0.05	0.835
race	-0.35	0.117	0.14	0.535	-0.26	0.259	0.14	0.552	0.25	0.268	0.08	0.729
Specific IgE*	0.10	0.663	0.51	0.019	-0.11	0.630	0.44	0.047	0.44	0.044	0.28	0.227
%Eosinophil	0.39	0.084	0.22	0.347	0.10	0.673	0.16	0.476	-0.04	0.849	0.00	0.993
# of positives SPT	0.29	0.201	0.50	0.021	0.27	0.231	0.71	0.000	0.65	0.001	0.70	0.000
# of SPT to pollens	0.28	0.222	0.51	0.019	0.48	0.029	0.70	0.000	0.60	0.004	0.65	0.002
SPT to Mt. Cedar	0.38	0.088	0.40	0.074	0.61	0.003	0.54	0.011	0.31	0.166	0.34	0.127
SPT to Cockroach	-0.06	0.780	-0.09	0.683	-0.20	0.379	0.04	0.851	0.08	0.736	0.11	0.625

r, rho value by Pearson correlation. DTSS was calculated as the difference between the maximum TSS and the baseline TSS, and computed from all the TSS values recorded in each of the 4 HDM exposures in ACC-I or ACC-II. * Class 0-4,

Fig. 1: Study design and TSS by stratification of participants by pollen and cockroach SPT reactivity



(A) Study phases. (B-D) Mean TSS (with standard deviation) of the 21 HDM+ individuals who completed all four study phases. Data shown are for **(B)** all subjects, and by SPT reactivity to **(C)** pollens and **(D)** German cockroach. rTSS values were recorded twice a day in the Run-in and observation phases as well as iTSS every 30 minutes in the ACC-I and ACC-II. P, pollen; CR, German cockroach.

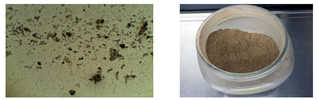
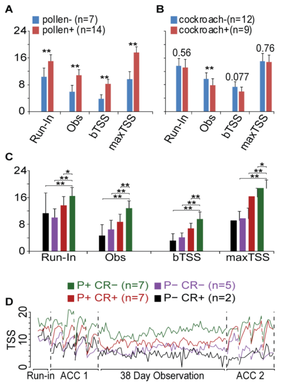


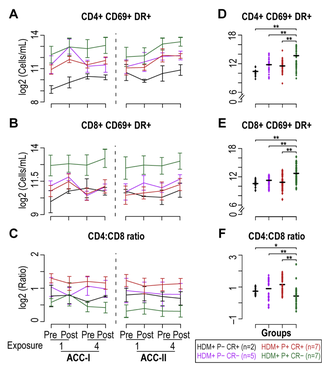
Figure 4: Microscopic and gross images of minced mite powder.

Fig. 2: TSS by pollen and cockroach SPT reactivity



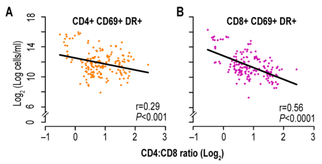
(A-C) Mean TSS (standard error of mean) from all the TSS values recorded in the run-in and observation phases, and the baseline and maximum TSS values recorded during each of the 4 exposures in ACC-I and ACC-II. Significance values by student t test. **(D)** Trajectory of the mean TSS stratified by SPT reactivity to pollens and German cockroach. *p<0.05, **p<0.01. C and D panels share the same key shown in panel D. P: pollen. CR: cockroach.

Fig. 3: T-cell activation and CD4:CD8 T-cell ratio by SPT reactivity



(A-C) Shown is the mean (SD) of (**A**) CD4+CD69+HLA-DR+ T-cells, (**B**) CD8+CD69+DR+ T-cells counts and (**C**) CD4:CD8 T-cell ratio according to 4 groups defined by the SPT reactivity to Pollen and German Cockroach. Data was derived before and after the first and fourth exposure in ACC-I and ACC-II. **(D-F)** Data derived from pooling the results from the 8 exposures shown in panels A-C. Dash, mean values. *p<0.05, **p<0.01. A-C and D-F panels share the same key shown below of F panel. P: pollen. CR: cockroach.

Figure 5: Inverse correlation between the CD4:CD8 T-cell ratio and levels of T-cell activation on CD4+ and CD8+ T-cells



Conclusions

- Higher numbers of positive SPTs for any aeroallergen and specifically to pollens and mountain cedar correlated positively with both the maximum iTSS in the ACC and the rTSS in the natural setting. The association was stronger in the ACC than that in the natural setting.
- The negative correlation between TSS and the SPT reactivity to German cockroach was less significant than the association with pollens.
- Stratification of the HDM+ participants by SPT reactivity to pollens and German cockroach identified four groups of patients with distinctive TSS phenotypes: the highest and least TSS were in *HDM+/pollen+/cockroach-* and *HDM+/pollen-/cockroach+* participants, respectively. The other two groups had an intermediate phenotype. Murine studies support the idea that cockroach exposure is protective against experimental asthma in mice [9].
- Those participants with the highest TSS defined by SPT to pollen and cockroach, i.e. *HDM+/pollen+/cockroach-* participants, also had the highest level of T cell activation and the lowest CD4:CD8 T-cell ratio compared to other groups. Thus, increased T-cell activation may be an important driver and biomarker of HDM disease severity.
- We suggest that by accounting for SPT reactivity, the use of an ACC reduces confounding factors and in turn, may increase a placebo-drug signal differential in clinical trials.

References

1. Platts-Mills T. et al. Middleton's Allergy: Principles & Practice. 7th ed. China: Elsevier Inc. 2009.
2. Devillier P, et al. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. Allergy 2011; 66:163-9.
3. Ellis AK, et al. Environmental exposure unit: a sensitive, specific, and reproducible methodology for allergen challenge. Ann Allergy Asthma Immunol 2013; 111:323-8.
4. Togias A. et al. Environmental exposure units: clinical trial design for validation. Bethesda: National Institute for Allergy and Infectious Diseases/National Institutes of Health; 2010. p. 1-12.
5. Jacobs RL, et al. Responses to ragweed pollen in a pollen challenge chamber versus seasonal exposure identify allergic rhinoconjunctivitis endotypes. J Allergy Clin Immunol 2012; 130:122-7 e8.
6. Jacobs RL, et al. Impact of Confounding Co-factors on Responses to Pollens During Natural Season Versus Pollen Challenge Chamber Exposure J Allergy Clin Immunol 2013; Dec 9. Epub ahead of print .
7. Horak F, et al. Efficacy and safety relative to placebo of an oral formulation of cetirizine and sustained-release pseudoephedrine in the management of nasal congestion. Allergy 1998; 53:849-56.
8. Horak F, et al. Effect of continuous allergen challenge on clinical symptoms and mediator release in dust-mite-allergic patients. Allergy 1998; 53:68-72.
9. Page K, et al. A TLR2 Agonist in German Cockroach Frass Activates MMP-9 Release and Is Protective against Allergic Inflammation in Mice. J Immunol 2009; 183:3400-3408.
10 .Calderon MA, et al. An evidence-based analysis of house dust mite allergen immunotherapy: a call for more rigorous clinical studies. J Allergy Clin Immunol 2013; 132:1322-36.