Evaluation of the effect of a nasal allergen challenge with Dermatophagoides farinae extract on nasal airway inflammation in allergic individuals, comparing e-cigarette users to cigarette smokers and non-smokers

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6.1. Protocol 1: Evaluation of the effect of a nasal allergen challenge with Dermatophagoides farinae extract on nasal airway inflammation in allergic individuals, comparing e-cigarette users to cigarette smokers and non-smokers.

a. Objectives and Purpose. The purpose of this pilot study is to evaluate allergen-induced inflammation in nasal lavage samples following ***Dermatophagoides farinae*** intranasal challenge in e-cigarette users, cigarette smokers, and non-smokers.

b. Patient inclusion and exclusion criteria.

We will recruit allergic subjects with or without mild asthma, ages 18-50 years, including **15** e-cigarette users, **15** tobacco cigarette smokers, and **15** non-smokers (classification described below). There will be no gender or ethnic restrictions. Allergy skin testing to *D. farinae* will be performed to confirm the presence of allergic sensitization. Only individuals sensitive to house dust mite will be selected for participation. Spirometry will be performed to determine the current level of lung function. Those with an FEV_1 (forced expiratory volume in the first second) of at least 80% predicted for age, gender, race, and height and an FEV_1 to FVC (forced vital capacity) ratio of at least .75will be eligible for the study. Subjects for this study will be both mild asthmatic and non-asthmatic healthy volunteers, ages 18-50years and to include both males and females. Our recruitment goal includes 45 volunteers with at least 50% female subjects and at least a 30% minority representation. It is anticipated that the demographics of our patient population will reflect those of the local university community. Many determinations (e.g. spirometry, allergy skin testing) are made as a part of a separate screening protocol, IRB #98-0799, which is performed to identify subjects for potential participation is experimental challenge protocols such as this one.

Inclusion criteria

1. Specific allergy to house dust mite *D. farinae* confirmed by positive immediate skin test response.

2. Subjects may be enrolled with mild asthma if an FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least .75 (without use of bronchodilator medications for 12 hours), consistent with lung function of persons with mild episodic or mild persistent asthma is demonstrated. For the purpose of this protocol, an asthmatic individual will be defined as having a) positive methacholine challenge with a provocative concentration of methacholine producing a 20% fall in FEV_1 (PC20 methacholine) with less than or equal to 10 mg/ml; OR b) physician diagnosed asthma with symptoms and chronic daily therapy consistent with the mild asthma.

3. Subjects will be classified as tobacco smokers, e-cigarette users, or non-smokers according to the following guidelines:

	Tobacco Smokers	E-cigarette Users	Non-smokers
Average cigs/wk	>35 cigs/week	<7 cigs/week	none
Average vapor puffs/day	<18 puffs/day	≥18 puffs/day	none
Hookah use	<30 minutes/week	<30 minutes/week	<30 minutes/week
Duration of use	If former heavy e-cig user, must be <18 puffs/day for ≥6 months	E-cig user ≥6 months; if former heavy tobacco user, must be <7 cigs/week for ≥6 months	Must be abstinent from tobacco or e-cig products for ≥6 months

4. Ability to withhold antihistamine medications for one week prior to baseline and allergen challenge visits.

5. Subjects must be able and willing to give informed consent.

Exclusion criteria

1. Any chronic medical condition considered by the PI as a contraindication to the allergen challenge study including significant cardiovascular disease, diabetes requiring medication, chronic renal disease, bleeding disorder, or chronic thyroid disease.

2. Physician directed emergency treatment for an asthma exacerbation within the preceding 12 months.

3. Use of systemic steroid therapy within the preceding 12 months for treatment of an asthma exacerbation.
4. Use of inhaled or nasal steroids, cromolyn or leukotriene receptor antagonists (Montelukast or Zafirkulast) within the past month (except for use of cromolyn exclusively prior to exercise).

5. Subjects who smoke marijuana or use illicit drugs will be excluded.

6. Use of daily theophylline within the past month.
7. Use of nasal medications that might alter the response to nasal allergen challenge including anti-inflammatory and anti-histamine agents within one week of challenge.

8. Inability to withhold inhaled or oral bronchodilator medications for 12 hours prior to allergen challenge.

9. Pregnancy or nursing a baby.

10. Women of child-bearing age who are not using dependable contraception (such as birth control pills, IUD, estrogen patches) or who are not completely abstinent.

11. Nighttime symptoms of cough or wheeze greater than 1x/week at baseline (not during a clearly recognized viral induced asthma exacerbation) which would be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma.

12. Exacerbation of asthma more than 2x/week which would be characteristic of a person with moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma.

13. Daily requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness) which would be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma. (Not to include prophylactic use of albuterol prior to exercise).

14. Viral upper respiratory tract infection or other acute inflammatory conditions of the nose or paranasal sinuses, such as sinusitis, within 4 weeks of challenge.

15. Any acute infection requiring antibiotics within 4 weeks of challenge.

16. Participating in an allergen inhalation study within 2 weeks of this challenge or use of any *other* investigational agent within the last 30 days.

17. Use of tricyclic antidepressants or beta-blockers.

18. Use of MAO inhibitors or any medications known to interfere with the treatment of anaphylaxis.

19. Subjects with a history of immunologic disease or undergoing immune suppression for cancer or other diseases.

c. Design of Study.

This study is designed to evaluate the effect of nasally applied *D. farinae* allergen extract on changes in nasal lavage, peripheral blood, and nasal epithelial cells in e-cigarette users compared to cigarette smokers and non-smokers. The principal endpoint is Δeosinophils/mL of nasal lavage fluid. Nasal allergen will be administered in a graded fashion distributing the dose equally between both nares with 100, 500 and 1,000 AU of *Der f* allergen extract delivered via nasal meter-dosed pump. Single-use nasal metered-dose pumps will be purchased from Professional Compounding Center of America, Houston, TX.

Secondary and exploratory endpoints will include nasal fluid cytokines, chemokines, markers of cellular activation, and mRNA from peripheral blood cells and nasal epithelial cells to examine gene expression profiles before and after nasal allergen challenge.

d. Dosage

Dermatophagoides farinae (*Der f*) allergen extract dilutions will be prepared in sterile saline by the University of North Carolina Hospitals Investigational Drug Service. It will be administered from a handheld nasal metered dose sprayer. The maximum cumulative dose that will be administered is 1600 AU of *D. farinae* extract distributed equally between both nares. Symptoms will be monitored and scored at regular intervals following allergen challenge. The challenge protocol is outlined in section "e" below.

e. Observations and Measurements

Observation Schedule:
Prior to any nasal inhalation challenge, subjects will undergo a physical examination of the ears, nose, throat (noting the uvula) and chest and will have an assessment of vital signs (temperature, pulse, respiratory rate, blood pressure), Total Nasal Symptom Score (TNSS) assessment and undergo spirometry to rule out acute illness prior to challenge. All female volunteers will undergo a urine pregnancy test. After adequate lung function has been documented, nasal allergen challenge will take place as outlined above. A physician will be immediately available during the challenge.

After nasal allergen challenge is complete, the subjects will be observed in CEMALB until venipuncture, nasal lavage (NL), epithelial lining fluid (ELF), and nasal epithelial cell (NEC) sampling are complete. Subjects will not be discharged from the CEMALB unit following challenge until FEV1 is within 90% of baseline values or higher. A physician will be on call following the challenge session for telephone consultation with the volunteer subject at any time following the nasal challenge.

Each subject will have four study visits:

Visit 0: Screening allergen challenge day studies:
1. Consent obtained
2. Urine pregnancy test for females of child bearing potential

3. Vital signs
4. Medical history (including medications) and physical exam (including oral exam, noting the uvula), and a smoking use questionnaire will be obtained.
5. Skin testing for *D farinae* sensitization (unless previously obtained).
6. Baseline spirometry
7. Lavage with normal saline to both nares to clear secretions and prepare for placement of allergen.
8. Total nasal symptom score (TNSS)
9. Peak nasal inspiratory flow (PNIF) measurement
10. Graded nasal allergen challenge using both nares with TNSS after each dose. Nasal allergen challenge will be provided in a graded dose fashion 0 AU, 100 AU, 500 AU and then 1,000 AU separated by 10 minutes. Dilutions will be provided by the University of North Carolina Hospitals Investigational Drug Service. Over 10 minutes, symptoms will be monitored and scored. Increasing concentrations of dust mite allergen dilution are deposited into the nose until the TNSS reaches a total of 8 or more (described below) and PNIF is reduced by at least 50%. An oral exam will occur prior to each dose. The cumulative allergen dose producing a TNSS of ≥ 8 and reduction in PNIF of ≥ 50% will be considered the provocative dose of allergen. Failure to achieve TNSS of ≥ 8 and reduction in PNIF of ≥ 50% will result in screen failure.
11. Subjects will be monitored for 90 minutes from the last dose of nasal allergen. Oral and upper airway exam, vital signs and spirometry will be performed at 30 and 60, and a physical exam at 90 minutes. At 90 minutes, if no significant findings on exam and no significant reduction in FEV1 (<10% reduction from baseline), the subject will be discharged.

Post Nasal Allergen Challenge Telephone Follow up (24 hours after Visit 0)

Subjects will be contacted for phone call follow-up 24 hours after allergen challenge (see phone script included in the study worksheets section)

Visit 1: Baseline visit (at least 7 days but within 2 months of screening)

1. Review of medical history including present medications
2. Obtain diaries detailing number of e-cig uses or number of cigarettes smoked per day (e-cig users and cigarette smokers only)
3. Urine pregnancy test for females of child bearing potential.
4. Nasal epithelial cell sampling of the right nare for mRNA to evaluate TLR2, TLR3, TLR4, TLR7, NLRP3, NLRP12, and CD14 expression)
5. Discharge to home.

Visit 2: Nasal Allergen Challenge visit (to occur at least 14 days after Visit 1)

1. Review of medical history including present medications
2. Obtain diaries detailing number of e-cig uses or number of cigarettes smoked per day (e-cig users and cigarette smokers only)
3. Urine pregnancy test for females of child bearing potential, and urine for NNAL and cotinine.

4. Physical examination (PE to include vital signs, assessment of the head, neck, chest, and baseline oral exam including the uvula by study MD)
5. Baseline TNSS and PNIF
6. Spirometry
7. Epithelial lining fluid (ELF) collection from both nares to measure baseline cytokines (ECP, Leukotrienes C4, D4, and E4; Prostaglandin D2, GM-CSF, Il-4, IL-8, IL-13, RANTES, Eotaxin, soluble CD14 (LPS-binding protein), TNF, IL1, IL6, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β)
8. Nasal lavage of both nares for baseline assessment of nasal fluid cellularity
9. Venipuncture for evaluation of baseline complete blood count and differential, Der f IgE levels, mRNA, cytokines, and flow cytometry for the following cell surface markers: CD11b, CD14, CD80/86, HLA-DR, TLR 4, FcεR1.
10. Nasal allergen challenge with the previously demonstrated provocative dose of allergen administered with equal distribution between the nares. An oral exam will occur prior to administration of allergen.
11. Post nasal challenge TNSS and PNIF measurements performed at 10, 30, and 60 minutes then hourly for 4 hours.
12. Post nasal challenge, vital signs, spirometry and oral and upper airway exam will be performed at 30, 60, and 90 minutes following allergen challenge. At 4 hours a physical exam will be performed along with vital signs and spirometry.
13. Nasal lavage, nasal epithelial cell sampling (from the left nare), ELF collection, and venipuncture will occur 4 hours after allergen challenge for measurements as noted above.
14. If any reduction in FEV1≥10% from baseline is seen during the initial 90 minutes post-allergen challenge, spirometry will be repeated prior to discharge. If spirometry is not significantly reduced (reduction of <10% from baseline), the subject will be discharged.

Post Nasal Allergen Challenge Telephone Follow up (24 hours after Visit 2)
Subjects will be contacted for phone call follow-up 24 hours after allergen challenge (see phone script below)

Discontinuation visit: (5-10 days after challenge)
1. Vital signs
2. PE (including oral exam)
3. Spirometry

Endpoints
The primary endpoint for this study is Δeosinophils/mL of nasal lavage fluid (post-allergen challenge minus pre-allergen challenge values), comparing e-cigarette users to cigarette smokers and non-smokers. A secondary endpoint will be ΔEosinophilic cationic protein (ΔECP), a marker of eosinophil activation. Exploratory endpoints as well as safety criteria for stopping involvement of an individual in the protocol or suspending the overall study are outlined below.

Exploratory Endpoints
Exploratory endpoints of interest from examination of *epithelial lining fluid (ELF)* and *nasal lavage fluid* collection include:

1. Cytokines and chemokines: Leukotriene C4, D4, and E4, PGD2, GM-CSF, IL-4, IL-8 IL-13, RANTES, Eotaxin, soluble CD14, LPS-binding protein, Dermatophagoides Farinae (Der F) specific and total IgE , TNF, IL1, IL6, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β).

Exploratory endpoints of interest from examination of *nasal epithelial cell* sampling include:

2. mRNA to evaluate gene expression profiles: TLR2, TLR3, TLR4, TLR7, NLRP3, NLRP12, and CD14 expression.

Exploratory endpoints of interest from examination of *peripheral blood* include:

3. Complete blood count and differential
4. mRNA from peripheral blood mononuclear cells (PMBCs)
5. Cytokine profile examined in nasal fluid samples
6. Flow cytometry for cell surface markers: CD11b, CD14, CD80/86, HLA-DR, TLR 4, FcεR1

Safety measures
Criteria for safety of a given individual following a specific challenge will include (failure of which would result in discontinuation of that subject from further study):

1. No greater than 10% decrease in FEV1 from pre-challenge values following a nasal allergen challenge to *D. farinae* allergen.

2. FEV1 must recover without treatment to within 85% of baseline within 6 hours of challenge without treatment, or if albuterol is employed the volunteer must recover to within 90% of baseline value within 20 minutes. All persons with a ≥10% decrease in FEV1 or those with respiratory symptoms will be given albuterol.

3. If albuterol therapy is needed more than three times within the first hour or more than 3 times during the observation period.

4. Requirement of oral corticosteroid treatment due to asthma exacerbation or other serious allergic reaction associated with challenge.

5. Evidence of upper airway obstruction, defined by the subject complaining of difficulty breathing, excessive salivation, changes in voice quality, and stridor. If an individual experiences evidence of upper airway obstruction, the subject will be treated with epinephrine via administration of an EpiPen device, as well as topical application of epinephrine if there is uvular involvement.

Criteria for safety within the entire protocol (failure of which would result in suspension of further study until consultation with the CBER of the FDA and the UNC IRB) will include the following:

1. No more than 20 % of patients will fail the individual safety criteria outlined above. As it is anticipated that 45 subjects will be recruited into this study, then 9 or more subjects who fail individual safety measures will result in suspension of further study until consultation with the CBER of the FDA.

2. No occurrence of any Serious Adverse Event

Subjects will be under direct supervision for at least 90 minutes post challenge and will have contact information for after-hours access to a study physician. In the event of an unexpected event, such as wheezing, urticaria or other allergic symptoms, the physician will follow the usual medical standard of care to treat the subject.

Confidentiality
Risks to subject confidentiality will be minimized by storing records with personal identifiers in an office in CEMALB which is locked when unattended by the study coordinators. All samples will be stored with codes only (no personal identifiers). The CEMALB is located in the US Environmental Protection Agency's Human Studies Facility on the UNC campus which has a security guard and limited access 24 hours/day, 7 days/week.

Efficacy Measure
The primary endpoint for efficacy of the nasal allergen challenge will be Δeosinophils/mL of nasal lavage fluid, comparing e-cigarette users to cigarette smokers and non-smokers.

Analysis Plan
For analysis of nasal eosinophil responses, we will use (i) descriptive analysis and (ii) two-sample comparison tests. Specifically, we will use the tabular and graphic methods to describe/explore the basic relationship among the groups. We will then follow up by comparing the Δ (post-allergen)-(baseline) responses of the e-cigarette and tobacco smoker groups using two-sample tests, either parametric (t test) or non-parametric (Mann Whitney U), depending on whether the normality assumption is met. We will transform the data to achieve normality if needed. These analyses will be repeated for comparisons of e-cigarette users to non-smokers and for tobacco smokers to non-smokers. In all analyses, criterion for significance will be $p \leq 0.05$. Dr. Haibo Zhou, the biostatistician for the CEMALB, will oversee all statistical analysis. To support the primary endpoint, we will compare the mean change in NELF eosinophilic cationic protein (ECP) at 4 hours post-allergen challenge from baseline between the e-cigarette users versus tobacco smokers, between the e-cigarette users versus non-smokers, and between tobacco

smokers versus non-smokers using ANCOVA to adjust for baseline NELF ECP. The NELF ECP AUC will be calculated using the trapezoidal rule.

Procedures to be Performed

Nasal allergen challenge procedure: Allergen dilutions will be prepared from a 30,000 AU/mL extract solution purchased from Greer® Labs and loaded into nasal actuator devices by a licensed pharmacist in the UNC Investigational Drug Service. Specific lot numbers will be recorded, and CBER will be informed of any lot extract changes. The subject will be instructed to inhale to total lung capacity (TLC). When at TLC, a single-use nasal metered dose pump will then be placed at the base of the subject's naris and activated delivering 100 uL of volume per spray, without an inhalation from the subject. After the correct dose is given, a timer will be set for 10 seconds. At the end of the time the subject will be asked to gently expel excess fluid from the nose into a cup which will be discarded. The technician will spray the solution sequentially distributing between both nares with 0 AU (glycerinated vehicle control), 100 AU (2 sprays or 200 uL of a solution of *Der F* extract of 500 AU/ml), 500 AU (2 sprays or 200 uL of a solution of *Der F* extract of 2500 AU/mL) and 1,000 AU of *Der F* allergen extract (2 sprays or 200 uL of a solution of *Der f* extract of 5000 AU/ml). Once the solution is delivered, the subject will be asked to exhale. The subject will NOT "sniff" the solution. TNSS will be assessed 10 minutes after each dose of the graded nasal dose challenge. Challenge will be stopped when a TNSS of 8 or greater and reduction in PNIF of at least 50% are achieved, or the maximal dose (1,000 AU) is administered.

Subjects will be attended for a minimum of 90 minutes following allergen challenge. An oral exam will be performed prior to each successive dose of allergen and at 30, 60 and 90 minutes post-allergen challenge.

Total Nasal Symptom Score (TNSS):

Nasal Allergen Symptom score (scale 0-none, 1-mild, 2-moderate, 3-severe):
Sneezing____, Congestion____, Nasal Itching_____, Rhinorrhea (Runny nose) ____,

The highest possible score will be 12 and the lowest is 0. A mild score for every criteria is 4, moderate is 8 and severe is 12.

Symptom score describer definitions:

Mild: Symptom minimally noticeable but would not ordinarily cause you to stop normal activities such as going to work or school and would not keep you from undertaking physical exercise.

Moderate: Symptom present but would not ordinarily cause you to stop normal activities such as going to work or school, although it might stop you from doing physical exercise ("working out")

Severe: Symptom clearly present and would cause you to consider not going to work or school or seek medical attention (such as calling a health care provider, going to student health, going to the emergency room, or taking medications for these symptoms).

Follow-up Symptom Score (administered by telephone for post-challenge observation):
1. On a scale of 0 to 3, with 0=no symptoms, 1=mild symptoms, 2=moderate symptoms, and 3=severe symptoms, please rate the severity of each of the following symptoms:

Sneezing (0-3)____
Congestion (0-3) ____
Nasal Itching(0-3) _____
Rhinorrhea(0-3)_____

2. Do you have any health concerns since we saw you on the day of the challenge?

3. Have you needed to see a doctor for any reason since your last visit?

4. Have you needed to use any over the counter medications?

5. Have you had any specific problems with nasal discomfort, cough, wheezing or have you needed any extra allergy or asthma medicines?

If the symptom score is 6 (out of 12), if any single symptom is scored at 3, or if the answers to questions 2, 3, 4, or 5 are "yes", then subjects will be offered the opportunity to come back to the laboratory for follow-up assessment.

Nasal lavage procedure*:*
Both nares will be lavaged. A standard amount of saline will be sprayed into each nare using a nasal meter dosed sprayer which delivers 100ul/actuation. Each lavage is comprised of eight sets of five sprays. Lavage fluid will be recovered by forceful expulsion of lavage fluid into a specimen cup immediately after each set of 5 actuations. Samples will then be transported on ice to the laboratory where slides for differential cell counts of uncentrifuged NLF will be made. The remainder of each specimen will be centrifuged and stored at -70º C until further analysis.

Nasal epithelial lining fluid (ELF) collection procedure:
ELF is obtained by spraying the nostril with 0.9% sterile, normal saline irrigation solution (about 100 µl per nostril) one time per nostril. One 10x55mm strip of filter paper, cut from Leukosorb paper (Pall Scientific, Port Washington, NY) on a laser cutter, is inserted into the anterior part of the inferior nasal turbinate of each nostril. The nostrils are clamped shut using a padded nose clip for two minutes. Strips are then removed from the nostril and collected in 1.5ml tubes. They are then stored in strips in a -20°C freezer until elution.

Venipuncture:
Venous blood will be obtained by venipuncture for various exploratory endpoints and for cotinine and NNAL levels to detect recent tobacco or e-cigarette use.

Spirometry and Peak Expiratory Flow (PEF) Measurement: Standard methodology conforming to the American Thoracic Society guidelines for measurement of spirometry and peak exploratory flow will be used.

Allergy Skin Testing:
Allergy skin testing is performed epicutaneously, examining wheal and flare reactions to histamine, a glycerol control, and various allergens. A Greer® Pick® device that has been immersed in a solution of allergen extract purchased from Greer® Laboratories is used to apply allergen epicutaneously by placing the device at a 45 degree angle to the skin and gently lifting up to create a skin prick. Reagents are obtained from Greer Laboratories and tests are performed as outlined for FDA approved procedures for evaluation of allergy. All testing instruments are single patient use. Each patient will undergo skin prick testing with the following allergen preparations: House Dust Mite (D. farinae), House dust mite p (*D. pteronyssinus*), Cockroach, Tree mix, Grass Mix, Weed Mix, Mold Mix 1, Mold Mix 2, Rat, Mouse, Guinea Pig, Rabbit, Cat and Dog. The allergens will be placed on the forearm. After 15 minutes, a research coordinator will compare the wheal size (a raised bump surrounded by red itchy skin) for each allergen to that of the negative and positive control (histamine). A positive skin test is defined by a 3 mm wheal greater than the saline control. For a volunteer to be considered non-allergic, the histamine control must be reactive and all others must be negative.

Nasal Epithelial Biopsy:
This is a non-invasive biopsy procedure performed to retrieve a small cluster of cells from each of the nasal cavities. For the nasal biopsy procedure, the subject will be seated comfortably in a straight-backed chair or reclining on an examination table with the head tilted as far back as possible while remaining comfortable. Subjects will be given no medication, sedation, or anesthesia for this procedure. A short, sterile plastic sampling device called a curette will be inserted into one of the nasal cavities and the surface of the nasal cavity will be stroked several times for approximately 5 seconds in order to obtain a small cluster of cells. Due to the potential for the biopsy itself to create a local inflammatory response, we will ensure that visit 2 occurs no sooner than 14 days after visit 1 to allow for dissipation of this inflammatory response before collection of nasal fluid samples. We will sample nasal epithelium from the right nare during visit 1 and from the left nare during visit 2 to ensure we obtain adequate epithelial samples on each occasion.

Table of Study Procedures
Procedures to be performed at various time points are listed in the following table and in the accompanying study worksheets.

	Screening day	24 hrs post challenge (phone)	Baseline Visit	Allergen Challenge day	24 hrs post challenge (phone)	Discontinuation visit
Consent	X					
Collect smoking diaries (if applicable)			X	X		
Review history/AE's	X	X		X	X	X
Follow up symptom score		X			X	X
Urine HCG (prior to challenge)	X		X	X		
Vital signs	X		X	X		X
Spirometry/PEF	X			X		X
Physical exam	X			X		X
Total nasal symptom score	X			X		
Peak nasal inspiratory flow rate	X			X		
Allergen challenge	X			X		
Nasal lavage				X		
Nasal epithelial cell collection			X	X		
Epithelial lining fluid collection				X		
Venipuncture	X			X		

Post Challenge Observations: Reporting

Within 24 hours after allergen challenge, each volunteer will be evaluated using the Follow up Symptom Score described above. If the symptom score is 6 (out of 12), if any single symptom is scored at 3, or if the answers to questions 2, 3, 4, or 5 are "yes", then subjects will be offered the opportunity to come back to the laboratory for follow-up assessment.

Study discontinuation visit:

Within 10 days of the final challenge dose, each subject will be asked to return for a study discontinuation visit. At that time temperature, pulse, systolic and diastolic BP, respiratory rate, FVC and FEV_1, and symptoms scores will be assessed and, if abnormal, medical evaluation as directed by the study physician will be undertaken.

Definition of an adverse event (AE) and Serious Adverse Event (SAE):

An adverse event for a given volunteer will be defined as failure of any of the safety criteria outlined in section "e". Additionally, minor upper respiratory tract infections occurring within 96 hours of the exposure will be considered adverse events. Other, non-specified clinical illnesses, which occur within 96 hours of each challenge, will also be reported as an AE. Any decrease in lung function or increase in symptom score, as outlined in section "e" will be considered an adverse event. Any symptoms that induce a

volunteer to seek medical attention from any provider within 96 hours of challenge will be considered an adverse event.

Adverse events will be graded using Common Terminology Criteria for Adverse Events v3.0, (CTCAE website at http://ctep.cancer.gov/reporting/ctcnew.html and http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx). Symptom scores will be graded as outlined elsewhere in this application.

The severity grades of AE's are defined as follows:
Grade 1: Mild AE
Grade 2: Moderate AE
Grade 3: Severe AE

A serious adverse event will be defined as any event that requires hospitalization or results in life threatening illness or injury, permanent (or likely to be permanent) illness or injury, or death if these events occur within 96 hours of challenge (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 96 hours of a challenge).

f. Monitoring and risk minimization

Risks to subjects: In previous studies performed in our laboratory (three published studies), which includes over 70 nasal allergen challenges in volunteers with mild allergic asthma, approximately half of these attained the 1000 AU dose (with a total cumulative dose of 1100 AU). The most commonly encountered (and anticipated) effect was increased nasal allergic symptoms. These were typically short term symptoms (resolved within 24 to 48 hours) which rarely required a short course of antihistamines. While these studies did not involve regular measures of lung function after nasal challenge, there were no complaints of increased bronchial symptoms and no subjects required inhaled bronchodilators or inhaled or oral corticosteroids. There has been no episode of systemic anaphylaxis or acute bronchospasm associated with nasal allergen challenge.

One subject had an episode of allergic uvulitis following nasal allergen challenge (discussed further in section 9, Previous Human Experience). We suspect that the sniffing that occurred with the challenge may have contributed to that risk, in addition to the subject having laid recumbent immediately after the nasal allergen challenge was finished. Therefore, in this study the subject will not sniff, rather the allergen will be delivered to the nose in such a fashion as to avoid deposition of the allergen in the pharynx. Additionally, subjects will be advised not to lie recumbent for 4 hours after the completion of the last challenge dose.

The most likely and significant risk to subjects will be increase in allergic rhinitis symptoms, including congestion, rhinorrhea, nasal itching and sneezing. Other possible, although unlikely, effects include increased cough, and increased occurrence and severity of bronchospasm. Hypothetical risks, which we have not

observed in our previous studies, include generalized allergic symptoms, including urticaria, flushing, hypotension and anaphylaxis.

Measures to minimize risk: Only people with allergic rhinitis and/or mild asthma, as characterized the NHLBI guidelines for the diagnosis and management of asthma, will be recruited for this project. Please note that our exclusion criteria for subjects include symptom or medication use criteria that would result in classification of an asthmatic as moderate or severe. Further, subjects will be deferred for challenge until 4 weeks after complete resolution of each of the following acute illnesses: viral respiratory tract infection, pneumonia or bronchitis requiring antibiotic therapy (must be off antibiotics and well for 2 weeks after the last dose of antibiotics, 4 weeks in the case of azithromycin due to its prolonged half-life), or acute illness resulting in fever. Also, unspecified illnesses, which in the judgment of the investigator increase the risks associated with allergen inhalation challenge, will be a basis for exclusion. As outlined above in section ***e***, all subjects will need to fulfill objective lung function and symptom criteria prior to initiating a specific challenge study.

A physician familiar with the protocol will be available for all challenge procedures. Emergency treatment with topical and intramuscular epinephrine, albuterol, oxygen, antihistamines and oral corticosteroids will be available to those patients who require such therapy. The human exposure laboratory is also equipped with an emergency "crash cart" with standard emergency medications, IV fluids and a defibrillator in the unlikely event of a medical emergency for any challenge study.

All subjects will be will be monitored as outlined in previous sections. A follow-up call will be made 24 hours after challenge to ensure that subjects are well, and all subjects will be provided with a contact telephone number for access to a study physician who is on call 24 hours/day. Subject may be withdrawn from the study at any point if the physician determines it is in the best interest of the subject.

Reporting of AEs and SAEs

All SAEs will be reported to the CBER of the FDA as well as to the UNC Biomedical IRB within 24 hours of recognition of the event. Adverse events will be reported to both the FDA and UNC IRB on no less than a quarterly basis, or when the protocol is completed. If criteria for suspension of the protocol are met, then the FDA and UNC IRB will be notified within 24 hours.

All subjects with a non-fatal SAE will be evaluated medically by a study physician, in concert with their own physician as appropriate. Likewise all subjects with an adverse event will be examined and evaluated by a study physician. All assessments will include the same lung function and vital sign assessments outlined for challenge observation. Other assessments will be undertaken as needed. Any unspecified event, which in the judgment of the PI of the study, constitutes an unusual, unexpected or prolonged event (greater than 96 hours) will be reported to both the FDA and the UNC IRB.

Curriculum Vitae for investigators are appended to this application.

6.2. Informed Consent

Investigators and study staff will explain all study procedures and the benefits and risks of the study to potential participants as part of obtaining informed consent. Subjects may withdraw their consent at any time during the study. If they withdraw from the study it will not impact the care they receive at UNC or its affiliated hospitals and clinics.

6.3. Investigators, Facilities, and Institutional Review Board

Role in project	*Name and Address*	*Title*
Principal Investigator	Michelle L. Hernandez, MD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Associate Professor of Pediatrics Chief Medical Officer, Center for Environmental Medicine, Asthma and Lung Biology
Co-investigator	Robert Tarran, PhD 7102 Marsico Hall The University of North Carolina CB#7248 Chapel Hill, NC 27599-7248	Associate Professor of Cell Biology and Physiology Director, UNC Center for Tobacco Regulatory Science and Lung Health
Co-Investigator	David B. Peden, MD, MS CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Professor of Pediatrics & Director, Center for Environmental Medicine, Asthma and Lung Biology
Co-Investigator	Allison J. Burbank, MD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Post-doctoral fellow UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology

Co-Investigator	Charity G. Duran, PhD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Post-doctoral fellow UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology
Co-Investigator	Chanchaldeep (Amika) K Sood, M.D. CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Post-doctoral fellow UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology

Facilities. Volunteers for these studies will be recruited, screened and undergo challenge procedures at the Center for Environmental Medicine, Asthma and Lung Biology, CB#7310, 104 Mason Farm Road, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7310. All necessary clinical research equipment, medical equipment, and laboratory equipment is located within the Center.

Institutional Review Board. This study and all changes will be reviewed and approved by the Biomedical IRB for the UNC School of Medicine, The University of North Carolina at Chapel Hill CB# 7097 720 Martin Luther King, Jr. Blvd. Bldg# 385, Second Floor Chapel Hill, NC 27599-7097