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IDENTIFICATION OF ALLERGIC ASTHMATICS REACTIVE TO *DERMATOPHAGOIDES FARINAE* (HOUSE DUST MITE) INHALATION

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Study Product:

Standardized house dust mite (*Dermatophagoides farinae*) allergen extract at 10,000 allergen units (AU)/mL provided by Greer Laboratories, Lenoir, NC.

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List of Abbreviations

AE: Adverse Event
AHR: Airway Hyperreactivity
AU: Allergen Units
AUC: Area Under the Curve
CBC: Complete Blood Count
CBER: Center for Biologics Evaluation and Research
CEMALB: Center for Environmental Medicine, Asthma and Lung Biology
CTRC: Clinical Translational Research Center
DerF: *Dermatophagoides farinae* (house dust mite)
ELISA: Enzyme-linked Immunosorbent Assay
ENO: exhaled nitric oxide
EPR: Early phase response
FEV_1: Forced Expiratory Volume in 1 Second
FRC: Forced Residual Capacity
FVC: Forced Vital Capacity
HCG: Human Chorionic Gonadotropin
HRV: Heart Rate Variability
IgE: immunoglobulin E
IL: Interleukin
LAIV: Live Attenuated Influenza Virus
LDMS: Lab Data Monitoring System
LPR: Late phase response
MDI: Metered Dose Inhaler
ML: Milliliters
MMHG: Millimeters of Mercury
NACL: Sodium Chloride
NO: Nitric Oxide
NSAIDS: Non-steroidal Anti-inflammatory Drugs
NSBR: Non-specific Bronchial Reactivity
PD20: provocative dose of allergen resulting in ≥20% fall in FEV_1
SAE: Serious Adverse Event
SOP: Standard Operating Procedures
TH1/TH2: T Helper Cell Type 1/2
TSLP: Thymic Stromal Lymphopoetin

1. Introduction

This document is a protocol of a human research study. This study is to be conducted according to US and international standards for Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background and Rationale

Asthma is an increasingly common chronic illness among children and adults (1), and allergen exposure is among the most common triggers for asthma exacerbations. Exacerbations of allergic asthma are characterized by an early phase response (EPR), mediated by release of preformed mediators like histamine from mast cells, and a late phase response (LPR) 3-7 hours later mediated by chemokines and cytokines that attract leukocytes such as neutrophils and eosinophils to the airways, increase mucus production, trigger airway smooth muscle contraction, and result in airway constriction and airway hyperreactivity (AHR). The LPR does not occur in the absence of an EPR. The LPR is thought to be predominantly responsible for the symptoms associated with acute exacerbations of allergic asthma and is often used as the measure of efficacy in trials of asthma therapeutics.

O'Byrne and others have used allergen challenge to understand the pathophysiology of asthma and screen asthma interventions (2-6). This technique has been extensively used to screen a number of new asthma agents in Phase IIa studies. This list includes anti-IL5 (7), anti-IgE (8, 9), anti-TSLP (10), and a number of other asthma agents (4, 11-15). In a recent review, Gauvreau et al (16) have noted that no currently available effective asthma agents failed to demonstrate a physiologically important effect in Phase 2a proof of concept studies using an inhaled allergen/LPR model in mild allergic asthmatics. Overall, allergen-inhalation challenge in mild asthmatic subjects has a very high negative predictive value and a reasonable positive predictive value for screening novel therapeutics for asthma for Phase III pivotal studies.

Our lab has taken a particular interest in targeting an inflammatory cytokine, Interleukin-1β, involved in both the early and late phase asthmatic responses to inhaled allergen in allergic asthmatics. In the lung, IL-1β is produced by numerous cell types (including epithelial cells, macrophages, neutrophils, eosinophils, and mast cells), where it signals through its receptor to induce transcription of pro-inflammatory genes (17-19). IL-1β is increased in bronchoalveolar lavage fluid from persons with symptomatic asthma vs. those with asymptomatic asthma (20); likewise, immunohistochemistry of bronchial biopsies of allergic asthmatics reveal increased expression of IL-1β in both bronchial epithelial cells and macrophages(18). Previous studies in animal and *in vitro* models demonstrate that IL-1β can directly impact three aspects of an airway inflammatory response: 1). granulocyte (neutrophil/eosinophil) recruitment (21, 22); 2). non-specific (23, 24) and allergen-specific airway reactivity (25, 26); and 3). production and clearance of airway mucous (27, 28). Supporting literature and our preliminary studies in human subjects further promote the study of IL-1 blockade for mitigating features of acute allergen-induced asthma exacerbation.

The role of IL-1 in allergen challenge models has not been fully defined. In a study examining 12 asthmatics allergic to *D. farinae* at our research center, we found that 9/12 asthmatics had a greater than 10% reduction in forced expiratory volume in 1 second (FEV_1) after inhaled dust mite challenge (29). These individuals were considered responders. It was notable that when comparing post-allergen levels of cytokines between responders and non-responders there was a much greater concentration of IL-1β in post-challenge sputum from responders vs. nonresponders, Furthermore, within the responders, post challenge IL-1β also significantly correlated with sputum eosinophil concentrations ($r=0.83$, $P<0.05$) and neutrophil concentrations ($r=0.89$, $P<0.05$) 24 hours after allergen challenge. These data suggest that IL-1β may play a role in both immediate airway hyperresponsiveness and the late phase recruitment of inflammatory cells (neutrophils and eosinophils) after inhaled allergen challenge.

Numerous IL-1 blocking agents are FDA-approved for conditions where the IL-1β pathway predominates disease pathophysiology such as in systemic juvenile idiopathic arthritis and the cryopyrin-associated periodic syndromes (30). Anakinra is a FDA-approved recombinant form of human IL-1 receptor antagonist (IL-1RA), a natural anti-inflammatory cytokine that competes with agonist binding to the IL-1 receptor, suppressing IL-1β and IL-1α signaling. With a fast onset of action, reaching peak concentrations in 3-7 hours, and a short 4-6 hour half-life, anakinra is an ideal candidate to test as a rescue treatment for acute asthma exacerbation.

The purpose of this study is to identify subjects with mild allergic asthma who experience a LPR after inhalation of *Dermatophagoides farinae* (DerF). Additionally, in order to better understand the role of IL-1β in allergen-induced airway inflammation, we will obtain induced sputum to determine if higher baseline sputum IL-1β concentrations or larger increases in IL-1β following allergen challenge impact non-specific airway hyperresponsiveness (via methacholine challenge), sputum granulocyte recruitment (neutrophil and eosinophil counts and exhaled nitric oxide (eNO), a marker of airway eosinophilia), sputum mucin content (MUC5AC, MUC5B), markers of systemic inflammation (inflammatory cytokines, absolute neutrophil or eosinophil counts in peripheral blood), or changes in expression of inflammatory or allergy-related genes. To this last point, little is known about the mechanisms contributing to response patterns in allergic asthmatics undergoing allergen challenge. We are particularly interested in gene expression changes occurring during the window of time between the EPR and LPR, as these expression changes may dictate whether or not a LPR occurs or to what extent it occurs (31, 32).

Once identified, these subjects will be eligible for participation in two randomized, double-blinded, placebo-controlled clinical trials of Anakinra vs placebo administered after allergen challenge for mitigation of features of an asthma exacerbation, including maximum drop in FEV_1 during the LPR, sputum granulocyte recruitment, mucous production and airway mucous clearance. The sample size needed to provide adequate power for the primary endpoint of each Anakinra study (maximum % fall from baseline in FEV_1 during the LPR) is **12 subjects per study**. Hence the goal of this screening protocol is to identify at least 25 subjects who will be eligible for enrollment in the

Anakinra protocols, which will be fully developed after our grant funding becomes available from the NHLBI. During the early phase of this project while we are developing the Anakinra protocol, our goal is to begin the process of screening allergic asthmatics for reactivity to DerF, specifically identifying those who exhibit both an EPR and LPR and can be later included in the Anakinra protocols.

1.2 House Dust Mite allergen for challenge

Standardized house dust mite (*D. farinae*) allergen extract at a potency of 10,000 AU/mL purchased from Greer Laboratories, Lenoir, NC.

1.3 Dose Rationale and Risk/Benefits

Dermatophagoides farinae (Der F) allergen extract dilutions will be prepared by the University of North Carolina Hospitals Investigational Drug Pharmacy in sterile saline by a licensed pharmacist and administered by a study coordinator from a DeVilbiss Ultraneb 646 ultrasonic nebulizer. Doses will be made up no more than 24 hours prior to administration. Doses of allergen will include 5 inhalations of allergen at concentrations of 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, 125, 250, 500, 1000, and 2000 AU/mL. Using an activation pressure of 20 lbs/sq. inch and duration of 0.6 sec of aerosolization, the nebulizer delivers 0.02 to 0.03 ml of solution. Each volunteer will inhale 5 breaths from the nebulizer at each concentration starting with saline control. Increasing (doubling) concentrations will be inhaled until the Forced Expiratory Volume in 1 Second (FEV_1) falls by $\geq$20% from the post saline value, the highest concentration has been inhaled, or the subject experiences discomfort or anxiety sufficient for the investigator or the subject to consider further testing unacceptable. The dose of allergen required to elicit a $\geq$20% drop in FEV_1 from the post-saline value at the screening visit will be defined as the provocative dose (PD20) and marks the onset of the EPR. The challenge will be stopped once the PD20 is reached. Following challenge completion, symptom scores and spirometry will be repeated every 10 minutes until 30 minutes, each 30 minutes until 2 hours, each hour until 10 hours, and again the next day. The subject will be discharged 10 hours following allergen challenge when clinically stable and when FEV1 is within approximately 10% of the pre-allergen baseline value. All subjects will be provided with rescue medications, including a short-acting bronchodilator inhaler with an aerochamber and oral prednisone 60 mg, at the time of discharge with detailed instructions for use in the event of symptoms related to bronchoconstriction and will return the following morning for reassessment.

This dosing regimen is based on that employed in two previous studies of mild allergic asthmatic volunteers [4,5] defining PD20. We have also conducted another study of 14 subjects in which the maximum dose employed was 2,560 AU/ml to establish a PD20 response [50].

At the doses proposed in this study, the most significant predictable risk to subjects as a result of allergen inhalation is development of immediate airway obstruction (bronchospasm) during the EPR, which would cause shortness of breath, cough and/or wheeze. This drop in FEV_1 during the EPR should be short lived (maximal 10 to 30 minutes after allergen), and reversible with medications such as beta agonists (albuterol).

This effect is similar to the effect of methacholine, which is an FDA-approved agent employed for the diagnosis of airway reactivity and asthma.

Other possible risks include development of a LPR to allergen inhalation. This occurs in approximately 50% of asthmatics challenged, consisting of similar symptoms described for the EPR. Since a key endpoint of this study is to identify which subjects have a LPR, albuterol for rescue prior to the 10 hour post challenge time point will only be employed if the FEV_1 does not show spontaneous improvement, and/or if the subject has distress necessitating rescue. All subjects will be given 4 puffs of albuterol at 10 hours after the inhaled allergen challenge.

2. Study Objective

Primary Objective: The purpose of this study is to identify subjects with mild allergic asthma who display a late phase response (LPR) after inhalation of *Dermatophagoides farinae* (DerF) for potential participation in two planned studies of interleukin-1 receptor antagonist (Anakinra) therapy against features of allergen-induced asthma exacerbation.

3. Study Design

3.1 General Design

This will be a non-blinded study to identify mild allergic asthmatics with measurable LPR to inhaled *Dermatophagoides farinae* allergen extract. The principal endpoint will be the presence of a LPR, defined as a decline in FEV1 of ≥15% from baseline values 3-10 hours after the onset of the EPR (which typically occurs within 30 minutes of allergen challenge).

Prior to enrollment, subjects will undergo a screening visit to establish eligibility (part of a separate screening protocol IRB#98-0799). Study visits will consist of a baseline visit to determine baseline values for study endpoints (Visit 1), a pre-allergen challenge visit to determine pre-challenge values for study endpoints (Visit 2), an allergen challenge visit (Visit 3), a visit 24 hours post-challenge to evaluate symptoms (Visit 4), and a final study discontinuation visit 5-10 days following allergen challenge. See Table in section 5.1.7.

3.2. Primary Study Endpoints

The principal endpoint of this screening inhaled allergen challenge protocol will be to identify those subjects who experience a greater than or equal to 15% drop in FEV_1 from baseline within 3-10 hours after the early phase response.

3.3 Secondary Study Endpoints

In addition to identifying subjects for participation in the planned studies of Anakinra vs placebo for mitigating features of asthma exacerbation, the additional purpose of this study is to determine if IL-1β concentrations in the sputum at baseline before challenge are predictive of key asthma outcomes following inhaled allergen challenge, specifically:

a) maximum drop in %FEV1 during LPR

b). Change in methacholine reactivity from baseline pre-allergen measurement to 24 hours after allergen challenge.
c). exhaled nitric oxide (eNO) level
d). induced sputum:
i. Granulocyte (neutrophils/eosinophils) numbers and percentages
ii. Mucins (secretion of MUC5AC and MUC5B determined by Western blot and ELISA)

Additionally, as exploratory endpoints, we will assess the effects of allergen challenge on markers of systemic inflammation and changes in inflammatory gene expression as described below:

a). **venipuncture** for assessment of systemic inflammatory markers (CBC with differential for absolute neutrophil count and absolute eosinophil count), pro-inflammatory cytokine levels such as IL-1β, IL-6, IL-8)
b). **gene array analysis** from blood and induced sputum at the baseline visit, again on the day of the challenge and, finally, at 24 hours post challenge. To identify signaling pathways potentially impacted by allergen challenge, we will use Nanostring® technology to measure differential expression of a panel of 594 immunology-related genes. This will be accomplished by collecting and isolating peripheral blood mononuclear cells from venous blood samples collected before allergen challenge, during allergen challenge, and 24 hours after allergen challenge.
c). **Heart rate variability**. We will place an ambulatory heart rate variabilty monitor on subjects before the challenge and monitor the heart rate for the duration of the post challenge monitoring, to investigate any acute cardiac changes.

3.4 Primary Safety Endpoints

3.4.1. Criteria to be met prior to initiation of allergen challenge

(Subjects not meeting these criteria on the morning of the challenge will not proceed with the allergen challenge).

1. FEV_1 of at least 80% of predicted and a ratio of FEV_1 to Forced Vital Capacity (FVC) of at least .70 (without use of bronchodilating medications for 8 hours, anticholinergic therapy (ipratropium) within 2 hours, or antihistamines within 5 days prior to challenge).

2. Baseline oxygen saturation of at least 95%.

3. No history of asthma exacerbation requiring systemic corticosteroid treatment, visit to an emergency room or hospitalization within 12 months of inhalation challenge.

4. No current history of requiring short-acting beta agonists more frequently than every 8 hours for asthma symptoms. Any use of albuterol in the week prior to allergen challenge will be reviewed by the study physician.

5. No history of viral respiratory tract symptoms within 4 weeks of challenge.

3.4.2. Criteria for safety within the entire protocol
(failure of which would result in subject withdrawal or suspension of further study) will include the following:

1. No occurrence of any Serious Adverse Event

2. No more than 20% of subjects will fail the individual safety criteria outlined below. As it is anticipated that 100 subjects will be recruited for this screening protocol, then 20 or more subjects who fail individual safety measures will result in suspension of further study until consultation with the CBER of the FDA. Additionally, in order to further monitor safety, we will assess this after completion of 20 subjects, and annually.

    a. Less than or equal to 40% decrease in FEV_1 or FVC from pre-challenge values following EPR or the LPR to *Dermatophagoides farinae* allergen (the target endpoint is a $\geq$20% decrease in FEV_1 from baseline during the EPR and a $\geq$15% decrease in FEV_1 from baseline during the LPR).

    b. If albuterol is employed as rescue, FEV1 must recover to within 90% of baseline value within 20 minutes.

    c. Oxygen saturation should remain $\geq$93% throughout the challenge period and must be no lower than 2% of baseline measure.

    d. Albuterol for rescue therapy should be required no more than three times within the first hour after challenge

    e. Albuterol for rescue therapy should be required no more than three times within 12 hours of discharge.

    f. FEV_1 should return to within 90% of baseline value at the study discontinuation visit which occurs within 10 days after allergen challenge.

    g. No more than 1 dose of oral corticosteroid (prednisone 60mg) should be required to treat an asthma exacerbation associated with inhalation challenge

4. Subject Selection

One hundred mild extrinsic (allergic) non-smoking asthmatics ages 18-45 will be recruited. There will be no gender or ethnic restrictions. Prior to enrollment in this

protocol, subjects will have undergone allergy skin testing as part of our IRB-approved general screening protocol. Only extrinsic asthmatics sensitive to house dust mite (*D. farinae*) will be selected for participation. Spirometry will be performed to determine the current level of lung function. Those with an FEV_1 of at least 80% predicted and an FEV_1 to FVC ratio of at least .70 off bronchodilators for at least 8 hours will have previously completed a standard graded dose methacholine bronchoprovocation challenge test to determine NSBR during the IRB-approved general screening protocol.

4.1 Inclusion Criteria

1. Age range 18-45 years, inclusive
2. FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least 0.7 (without use of bronchodilator medications for 8 hours or long acting beta agonists for 24 hours), consistent with lung function of persons with no more than mild intermittent or mild persistent asthma.
3. Physician diagnosis of asthma
4. Allergic sensitization to house dust mite (*D. farinae*) as confirmed by positive immediate skin prick test response
5. Negative pregnancy test for females who are not s/p hysterectomy with oophorectomy or who have been amenorrheic for 12 months or more.
6. Oxygen saturation of >94% and blood pressure within the following limits: Systolic between 150-90 mmHg and Diastolic between 90-60 mmHg.

4.2 Exclusion Criteria

1. Clinical contraindications:
    a. Any chronic medical condition considered by the PI as a contraindication to participation in the study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, history of chronic infections or immunodeficiency.
    b. Physician directed emergency treatment for an asthma exacerbation within the preceding 12 months.
    c. Exacerbation of asthma more than 2x/week which could be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma.
    d. Daily requirements 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).
    e. Viral upper respiratory tract infection within 4 weeks of challenge.
    f. Any acute infection requiring antibiotics within 6 weeks of exposure or fever of unknown origin within 6 weeks of challenge.
    g. Severe asthma
    h. Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.

i. Cigarette smoking >1 pack per month
j. 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.
k. Allergy/sensitivity to study drugs or their formulations
l. Known hypersensitivity to methacholine or to other parasympathomimetic agents
m. History of intubation for asthma
n. Unwillingness to limit coffee, tea, cola drinks, chocolate, or other foods containing caffeine after midnight on the days that methacholine challenge testing is to be performed.
o. Unwillingness in females to use reliable contraception if sexually active (IUD, birth control pills/patch, condoms).

2. Pregnancy or nursing a baby. Female volunteers will be asked to use effective birth control (stable regimen of hormonal contraceptive use for at least 3 months, intrauterine device placement, tubal ligation or endometrial ablation for at least 3 months through at least one week after study completion) and will provide a urine sample to test for pregnancy on study days. If the test is positive or the subject has reason to believe she may be pregnant, she will be dismissed from the study. Women who have been amenorrheic for 12 months may participate.

3. Usage of the following medications:
    a. Use of systemic steroid therapy within the preceding 12 months for an asthma exacerbation. All use of systemic steroids in the last year will be reviewed by a study physician.
    b. Subjects who are prescribed daily inhaled corticosteroids, cromolyn, or leukotriene inhibitors (Montelukast or Zafirlukast) will be required to discontinue these medications at least 2 weeks prior to their screening visit.
    c. Use of daily theophylline within the past month.
    d. 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).
    e. Use of any immunosuppressant therapy within the preceding 12 months will be reviewed by the study physician.
    f. Receipt of LAIV (Live Attenuated Influenza Vaccine), also known as FluMist®, or any other live viral vaccine within the prior 30 days, or any vaccine for at least 5 days
    g. Use of beta blocking medications
    h. Antihistamines in the 5 days prior to allergen challenge

i. Routine use of NSAIDs, including aspirin.

4. Physical/laboratory indications:
    a. Abnormalities on lung auscultation
    b. Temperature >37.8 C
    c. Oxygen saturation of <94%
    d. Systolic BP>150 mmHg or <90 mmHg or diastolic BP>90 mmHg or <60 mmHg

5. Inability or unwillingness of a participant to give written informed consent.

5. Study Procedures

5.1. Schedule of Events

5.1.1. Visit 1: Baseline visit (within 12 months of completing screening protocol IRB#98-0799)

1. Consent will be obtained
2. Review of subject's medical history and current medications
3. Vital sign measurements (temperature, pulse, respiratory rate, blood pressure), oxygen saturation, and symptom scoring
4. Urine pregnancy test for women of child bearing potential
5. eNO
6. Spirometry
7. Venipuncture for CBC with Diff, Total and der f IgE, cytokines, and gene expression
8. Physical exam of the ears, nose, throat and chest
9. Sputum induction

5.1.2. Visit 2: 24-48 hours prior to challenge visit (at least 2 days but less than 2 weeks after the baseline visit)

1. Review any change in medical status since last visit
2. Vital signs, oxygen saturation, and symptom score
3. Urine pregnancy test
4. eNO
5. Spirometry
6. Physical exam of the ears, nose, throat and chest
7. Methacholine challenge

5.1.3. Visit 3: Allergen challenge day

1. Review any change in medical status since last visit
2. Vital signs, oxygen saturation, and symptom score
3. Spirometry
4. eNO

5. Placement of HRV electrodes
6. If above measures are acceptable, allergen challenge will be performed as described.
    a. If subject is asymptomatic and FEV1 drops <10% at the completion of allergen challenge, subjects will be monitored for 2 hours post challenge and will be discharged following post-challenge venipuncture with rescue medications, including albuterol with aerochamber and oral prednisone 60 mg, instructions for use of these medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. Subject will resume protocol beginning with Study visit 4 detailed below.
    b. Any subject whose FEV1 drops ≥10% will stay for the 10 hour post-allergen monitoring period as outlined below.
7. Post-challenge monitoring of vital signs every 10 minutes for the 1st hour, then every 30 minutes until 2 hours post-challenge, then hourly until 10 hours post-challenge
8. Spirometry when vital signs are obtained
9. eNO hourly between 2 and 10 hours post-challenge
10. Venipuncture for CBC with diff, cytokines, and gene expression 10 hours post-challenge
11. All subjects will receive 4 puffs of albuterol 10 hours post-challenge. Spirometry will be performed 15 minutes after albuterol if post-challenge FEV1 at 10 hours was not approximately within 10% of baseline.
    a. Albuterol will be repeated every 20 minutes up to three doses if repeat FEV1 is not within approximately 10% of baseline value or if subject remains in distress.
    b. If needed, after three inhaled albuterol treatments, additional medications, including nebulized albuterol and atrovent and oral prednisone 60mg, will be given per physician discretion.
12. Subjects will be discharged 10 hours following allergen challenge when clinically stable and when FEV1 is within approximately 10% of baseline FEV1. HRV monitoring will be discontinued prior to discharge. At discharge, all subjects will be provided with an albuterol inhaler and aerochamber, a single dose of oral prednisone 60 mg, clear instructions for use of rescue medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. Subjects will reside overnight at their home if their residence is within 10 miles of UNC or a local hotel within 10 miles of UNC.

5.1.4. Visit 4: 24 hours post challenge

1. Vital signs, oxygen saturation, and symptom score
2. eNO
3. Spirometry
4. Venipuncture for CBC with Diff, gene expression, cytokines
5. Methacholine challenge
6. Sputum induction

5.1.5. Post Challenge Observations/Reporting

1. Subjects will be contacted for phone call follow-up 24 hours after post-challenge sputum induction (see phone script included in the study worksheets section)
2. Subjects who require treatment with prednisone will be contacted by phone within 24 hours to assess asthma symptoms.
3. Any subject who requires a dose of steroids will return to the lab 48-72 hours after the steroid dose for evaluation of symptoms, spirometry and a physical exam to assess the need for further steroid treatment.
4. Each volunteer will be given a symptom scoring sheet for each day up to 96 hours (4 days) after challenge (see symptom scoring sheet in section 5.2.7.).

5.1.6. Study discontinuation visit within 10 days of the final challenge dose:

1. Vital signs, oxygen saturation, and symptom score
2. eNO
3. Spirometry
4. If any findings are abnormal, medical evaluation as directed by the study physician will be undertaken.

5.1.7. Table of Study Procedures

Procedures to be performed at various time points are listed in the following table.

	baseline	24-48 hrs pre-challenge	allergen challenge day	24 hrs post-challenge	discontinuation visit
consent	X				
review history/AE's	X	X	X	X	X
Concomitant meds	X	X	X	X	X
urine HCG	X	X	X		
vital signs	X	X	X	X	X
spirometry	X	X	X	X	X
physical exam	X	X			
Methacholine challenge		X		X	
sputum induction	X			X	
symptom score	X	X	X	X	X
allergen challenge			X		
overnight in CTRC			X		
Venipuncture	X		X	X	
Exhaled nitric oxide	X	X	X	X	X
HRV			X		

5.2. Protocols for Measurements and Observations

5.2.1. Allergen challenge procedure:

Immediately prior to allergen challenge, determination of adequate lung function without use of bronchodilating medications for 8 hours will be made via spirometry with FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least .70, consistent with lung function of persons with mild intermittent or mild persistent asthma being considered minimal acceptable lung function.

Doses will be administered from a DeVilbiss 646 nebulizer as previously described. Each volunteer will inhale 5 breaths from the nebulizer at each concentration starting with saline control. After administration of the control diluent, concentrations of allergen will be administered starting with solutions of zero (saline), 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, 125, 250, 500, 1000, and 2000 AU/ml via nebulizer. During the inhalation challenge the subject will be under direct observation of a physician experienced in treating asthma. Epinephrine and diphenhydramine hydrochloride for intramuscular administration and albuterol for inhalation will be immediately available. The challenges will be performed in the Clinical and Translational Research Center (CTRC) of the University of North Carolina at Chapel Hill. Full emergency equipment and nursing personnel are immediately available at this location.

The FEV_1 will be measured prior to and 10 minutes after each aerosol inhalation. Starting with the control (saline diluent) solution, increasing concentrations of allergen will be inhaled at 10-minute intervals starting with 0.25 AU/ml. The FEV_1 measurement obtained after inhalation of the control solution (saline) will be considered the baseline value. If the FEV1 has declined by less than 10% of baseline after a given concentration of allergen is inhaled, the next higher concentration of allergen will be given. If after inhalation of an allergen dose, the FEV_1 declines by $\geq$10% but <20% from the baseline FEV1 measurement, spirometry will be repeated every 5 minutes for 15 minutes or until a clear nadir in the decline has been reached. If the nadir after 15 minutes is a decline in FEV1 of less than 10% of baseline, the next higher concentration of allergen will be given.If, however, the nadir after 15 minutes is a decline in $FEV_1 \geq 10\%$ but $< 20\%$ from the baseline measurement, the previous allergen dose will be repeated. . To prioritize the safety of subjects and to prevent severe or precipitous decline in lung function, decisions to repeat doses of allergen (rather than escalate dosing) or perform additional spirometry measurements will be made in real time at the discretion of the supervising physician, who will be physically present throughout the allergen challenge procedure. Once a decline in FEV of $\geq$ 20% is reached, the challenge will be stopped. These activities are summarized in **Figure 1**. Once the challenge is stopped, FEV_1 will be monitored every 10 minutes for the first hour, then every 30 minutes until 2 hours post-challenge, and then hourly until 10 hours post-challenge.

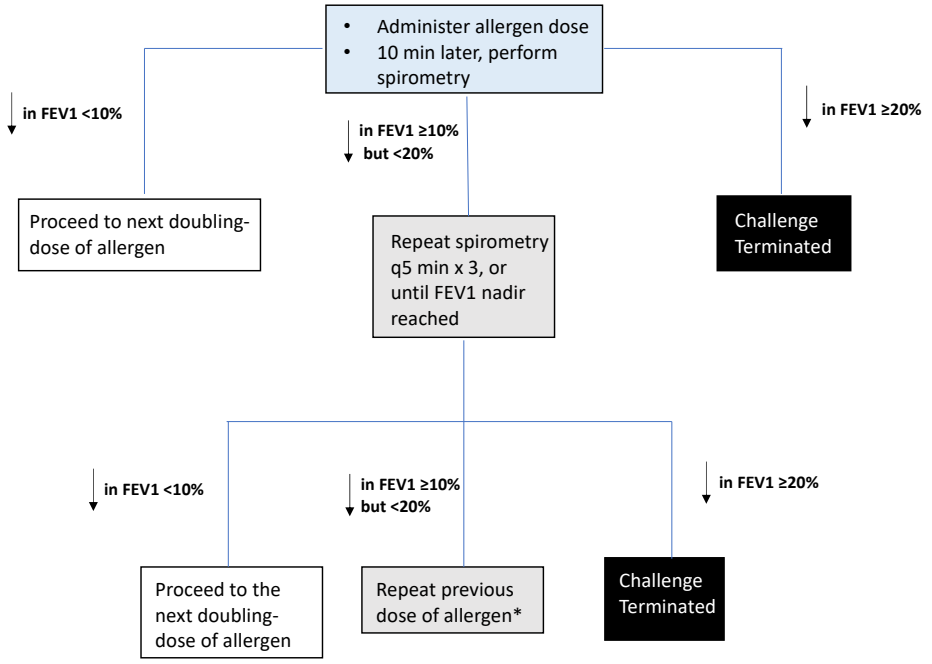

Figure 1. Inhaled allergen challenge schematic. *At discretion of supervising physician

Oxygen saturation by pulse oximeter will be measured during the challenge and with each spirometric evaluation. The physician responsible for the study will determine if any medication is necessary based on the subject's lung function and symptoms. Vital sign measurements including pulse oximetry evaluation will continue to be performed at hourly intervals during and for 10 hours after the exposure. Oxygen saturation by pulse oximeter will be measured during the challenge and with each spirometric evaluation. All subjects will receive 4 puffs of albuterol 10 hours after allergen challenge. If clinically stable and if FEV1 is approximately within 10% of baseline prechallenge FEV1, subject will be discharged with an albuterol inhaler with aerochamber and a single oral dose of prednisone 60 mg, clear instructions on use of medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. The subject will reside in either a local hotel within 10 miles of UNC or their home if their residence is within a 10 mile radius of UNC.

If the subject has wheezing on physical examination the morning after the inhaled allergen and/or has an FEV1 that is ≥15% from baseline measures (indicating that they are still experiencing a late phase response), they will be a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. These subjects will be contacted by phone within 24 hours of receiving the dose to assess asthma symptoms and will be evaluated in person within 48-72 hours of the dose to monitor FEV1 and to assess the need for continued therapy.

5.2.2. Hypertonic Saline Induced Sputum procedure:

Prior to sputum induction, subjects will receive 4 inhalations of albuterol from an MDI attached to a spacer device. FEV1 and FVC will be measured after this step to determine the post-bronchodilator baseline FEV1 and FVC values. Next, an ultrasonic nebulizer filled with 20 cc of 3% hypertonic saline (inhalation grade for respiratory use only, 3% NaCl) will be set to the maximum output setting and turned on. The subject will be instructed to latch his/her mouth onto the nebulizer mouthpiece and breathe normally (i.e., tidal breaths) for 7 minutes as the saline is nebulized through the mouthpiece in a jet stream and inhaled. The nose will not be occluded for this procedure. The subject will be encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower airways (i.e. not from the back of the throat) is ready for expectoration. Prior to expectoration, subjects will be asked to blow their nose, rinse their mouth with water, and clear their throat to avoid the inclusion of non-airway fluid samples. The sample will be expectorated into a sterile specimen jar and capped.

Following the measurement of FEV_1 after the first 7 minute inhalation period, the concentration of saline will be increased from 3% to 4%, provided the FEV_1 decrement is < 10% from the post-bronchodilator value. If the FEV_1 falls between 10-20% of the post bronchodilator value, the test will proceed but the concentration of saline will remain the same. If the FEV_1 falls by > 20% or if troublesome symptoms occur, the nebulization will be discontinued, and albuterol will be immediately available if necessary to relieve symptoms. The same procedure will be followed for the final 7 minute inhalation period using 5% hypertonic saline provided the FEV_1 safety parameters described above have been met. The nebulization is stopped after a total of 21 minutes or earlier if a sputum sample of good quality is obtained (i.e. visible sputum plugs).

5.2.3. Spirometry:

Standard methodology conforming to the American Thoracic Society guidelines for measurement of spirometry will be used.

5.2.4. Methacholine Challenge:

This test measures the responsiveness of the airways to a standard cholinergic bronchoconstriction agent. Methacholine challenge testing is a standard clinical procedure to determine airway reactivity in patients with known or suspected airway disease. Subjects will be asked to limit caffeine on the day of testing. Subjects will inhale 5 breaths from the nebulizer at each concentration starting with saline control. Each breath is started from resting end-expiratory lung volume (Forced Residual Capacity) and continued until a maximum inhalation is reached. Increasing (doubling) concentrations are inhaled until the FEV_1 falls by at least 20% from the post saline value, the highest concentration has been inhaled, or the subject experiences discomfort or anxiety sufficient for the investigator or subject to consider further testing unacceptable. Methacholine concentrations will be 0.075, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/ml. If subjects experience symptoms that do not rapidly and spontaneously remit at the end of the procedure, he/she will receive 2 puffs of an inhaled bronchodilator (albuterol) MDI. If needed an additional 2 puffs or a nebulizer treatment with standard dose albuterol (0.083mg) will be given. The subject will not be discharged until spirometry levels return to baseline.

5.2.5. Venipuncture:

Blood will be drawn to evaluate a complete blood count with differential and also to determine what genes may play a role in a person's response to allergen challenge. At the time of each venipuncture, up to 50 cc will be drawn, and the total for the study will be less than 150 cc.

5.2.6. Exhaled nitric oxide measurement:

We will measure the amount of nitric oxide (NO) present in orally expired air. An increased concentration of NO in exhaled air may be found in normal persons with acute inflammation. Thus, measurement of the concentration of NO in expired air may be useful as an indirect assessment of airway inflammation. Lung production of NO will be measured by asking subjects to exhale briefly into a mouthpiece.

5.2.7. 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:

Waking up at night due to cough (0-3): _____
Shortness of Breath (0-3): _____
Cough at rest (0-3): _____
Cough with Exercise (0-3): _____
Wheeze at Rest (0-3): _____
Wheeze with Exercise (0-3): _____

Total_____

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 cough, wheezing, or needed any extra allergy or asthma medications?

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 the subject will be offered the opportunity to come back to the laboratory for follow up assessment by a study physician.

6. Statistical Plan

6.1. Sample Size Determination

Sample size was calculated based on the sample required for our upcoming randomized placebo-controlled crossover studies of anakinra for mitigating features of allergen-induced asthma exacerbation. We hypothesize that anakinra treatment reduces the LPR, as assessed by the change in maximum percentage fall in %FEV1 during hours 3-10 following allergen challenge. We will be developing two anakinra treatment clinical trials, each of which will incorporate a crossover study design: one will provide anakinra treatment (v. placebo) immediately after the EPR, and one will provide anakinra treatment (v. placebo) at the onset of the LPR (i.e. 3 hours after the EPR).

We calculated the sample size for the anakinra treatment protocols using a model of inhaled allergen challenge similar to what we propose in this project (69) based on a paired t test. The maximal drop in %FEV1 during the LPR was 25.6% $\pm$ 9.8%(SD). To detect a 50% attenuation of the drop in %FEV1 during the LPR (12.8%) by anakinra v. placebo treatment, assuming that group-wise standard deviations are similar (10.28% for anakinra; 9.8% for placebo), with β=0.9 & α=0.05, a sample size of 6.4 would be required for each anakinra protocol. We estimate that 25% of subjects will require rescue short-acting beta agonist treatment during the EPR or the LPR, and will additionally account for a 25% dropout rate for a crossover study, inflating our required sample size to at least **N=12** for each anakinra treatment protocol.

Previous studies of inhaled allergen challenge models in allergic asthmatics have found that 50-70% of subjects who undergo challenge will experience an EPR (33, 34). Gauvreau et al (16) reported that >75% of *D. farinae* challenged volunteers who display an EPR will also have a LPR. Using a more conservative estimate of 50% of our screened volunteers with a EPR and 50% of those with a LPR, we will screen up to 100 subjects to identify at least **25** LPR subjects eligible to enroll into the anakinra treatment studies. Screening will stop following identification of 25 LPR subjects.

6.2. Statistical Methods

Our analytical plan was developed with Dr. Zhou, the biostatistician for the CEMALB who will oversee all statistical analysis. In all analyses, criterion for significance will be $p \leq 0.05$.

Our primary endpoint is identification of subjects who achieve a $\geq$15% fall in FEV_1 during hours 3-10 following allergen challenge. We will subtract the pre-allergen challenge %FEV1 value from the post-allergen %FEV1 value to make this determination.

For analysis of secondary endpoints, we will first use linear regression to determine if there is a linear relationship between baseline sputum IL-1β concentration (pg/mL) and asthma outcomes (maximum fall in %FEV1 during LPR, methacholine reactivity defined by the dose required to produce a $\geq$20% drop in FEV1, sputum granulocyte content both in terms of % of total cell count and cells/mg of sputum, exhaled nitric oxide concentration) following allergen challenge, adjusting for potential confounders. The point estimate and confidence interval will be reported for the effect of baseline sputum IL-1β on asthma outcomes. If the normality assumption is violated or there exists a non-linear relationship, we will calculate the Spearman correlation coefficient between baseline sputum IL-1β concentration and asthma outcomes.

For analysis of exploratory endpoints, we will use two sample comparison tests to compare systemic inflammatory markers and gene expression levels before and after allergen challenge. We will use two-sample t tests or Mann Whitney U tests depending on whether the normality assumption is met. The point estimate and confidence interval will be reported for mean changes. We will transform the data to achieve normality if needed.

As we have considered additional individuals in our sample size calculation, we do not anticipate missing data will be a major issue affecting our study power.

6.3. Data Management

Study coordinators responsible for efforts and computations for database management will include Carole Robinette, MS; Martha Almond, RRT; and Katherine Mills, BA. Data will be initially collected by study coordinators on paper documents. Symptom questionnaires will be completed by the subject and collected by the coordinator. Clinical data such as health history and smoking history will be collected by study staff as interview or directly measured, such as vital signs, spirometry, etc. All paper forms (source documentation) is maintained by a study coordinator or PI in a binder, 1 binder per subject. All data will be entered into REDCap by a study coordinator, within 2 weeks of collection and will be verified by a 2nd person within 4 weeks to ensure accuracy and completeness. REDCap is a secure web application developed at Vanderbilt University and can be used to collect virtually any type of data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments). It is specifically geared to support online or offline data capture for research studies and operations. Missing data will be noted and a comment made in the database to explain the missing data. Time stamps will be used when appropriate. At minimum, the visit number will be indicated.

Biological samples collected by coordinators will be delivered to the CEMALB lab by the coordinators in properly labeled containers. On receipt in the lab, samples are initially entered into a software program called LDMS (Lab Data Management System) which tracks samples. Processing of these samples is done per the SOP maintained in the CEMALB quality assurance plan, as some samples are processed immediately and others are processed in batches at later time points. This data is entered into REDCap at appropriate intervals, based on sample analysis.

We will use the data dictionary or REDCap codebook as our course for data codes. All data will be collected by qualified staff using CEMALB SOPs and will be entered into REDCap. Once the data is entered and confirmed by a 2nd person, it will be considered "locked" and will not be changed without documentation with the reason for the change. REDCap limits and monitors who can change data, a feature developed to ensure data integrity.

7. Monitoring and Risk Minimization

7.1. Definition of 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 3.4.2. Additionally, minor upper respiratory tract infections occurring within 10 days of the exposure will be considered adverse events. Other, non-specified clinical illnesses, which occur within 10 days of each challenge will also be reported as an AE. Specific potential adverse events which may occur with allergen challenge include the following which are defined in the Common Terminology Criteria for Adverse Events developed by the NCI: Allergic Reaction, Cough, Dyspnea, Urticaria, Syncope, and Voice Changes

Also, any decrease in lung function or increase in symptom score, as outlined in section 3.4.2. will be considered an adverse event. Failure of a total symptom score to return to no greater than 6 above baseline within 10 days will be considered an adverse event. Any symptoms that induce a volunteer to seek medical attention from any provider within 10 days 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 (CTCAE Grade 4), or death (CTCAE Grade 5), if these events occur within 10 days of challenge (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 10 days of a challenge).

7.2. Risk to Subjects

At the doses proposed in this study, the most significant predictable risk to subjects as a result of allergen inhalation is development of immediate airway obstruction (bronchospasm) during the EPR, which would cause shortness of breath, cough and/or wheeze. This drop in FEV_1 should be short lived (maximal 10 to 30 minutes after allergen) and reversible with medications such as beta agonists (albuterol). This effect is similar to the effect of methacholine, which is an FDA-approved agent employed for the diagnosis of airway reactivity and asthma.

Other possible risks include development of a LPR to allergen inhalation. This occurs in approximately 50% of asthmatics challenged, consisting of similar symptoms described for the EPR. Since an important secondary endpoint of this study is to identify which subjects have a late asthmatic response, albuterol for rescue prior to the 10 hour post challenge time point will only be employed if the FEV_1 does not show spontaneous

improvement, and/or if the subject has distress and requests rescue. All subjects will be given 4 puffs of albuterol 10 hours post-challenge with additional doses given based on FEV1 and subject distress.

Sputum induction may induce cough, chest tightness or bronchospasm, and albuterol will be immediately available.

Venipuncture carries a risk of hematoma, and this procedure will be performed only by trained staff members.

There is no risk to measurement of eNO since subjects are simply asked to exhale into a mouthpiece.

7.3. Measures to Minimize Risk

Only clinically healthy volunteers will be recruited for this project. 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 4 weeks after the last dose of antibiotics), 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 3.4.1., all subjects will need to fulfill objective lung function and symptom criteria prior to initiating the challenge study. Methacholine challenge is employed prior to the allergen challenge to ensure that the subject has a repeatable, within one-doubling dose response to the methacholine (compared to the previous methacholine challenge performed during the separate general screening protocol IRB#98-0799) to help rule out an exacerbation prior to the allergen challenge.

Female subjects of child-bearing potential will undergo urine HCG testing for pregnancy within 48 hours prior to administration of any challenge.

A physician familiar with the protocol will be available for all challenge procedures. Emergency treatment with albuterol (via MDI or nebulizer), oxygen, oral corticosteroids as well as epinephrine (subcutaneously or intramuscularly) and diphenhydramine (for intramuscular injection or intravenous administration) will be available to those subjects who require such therapy. An emergency “crash cart” with standard emergency medications, IV fluids and a defibrillator are is also readily available in the UNC CTRC as well as at CEMALB in the unlikely event of a medical emergency during any challenge or study visit.

If the covering physician feels that the subject’s respiratory status is such that providing an induced sputum sample would place them at increased risk for significant bronchospasm, we will prioritize spirometry assessments (as part of routine clinical care) and not obtain induced sputum samples for that subject.

All subjects will be monitored in the CTRC for 10 hours following allergen challenge as outlined in previous sections. Upon discharge to either the subject's home or local hotel, subjects will be provided with an albuterol inhaler with aerochamber and a single dose of oral prednisone 60 mg, clear instructions for use of these medications in the event of bronchoconstriction, and the contact information for the on-call physician. In the event of post-challenge bronchoconstriction that does not improve with albuterol, subject will be instructed to present to the UNC emergency department for further evaluation and management. Subjects will also be assessed the morning after the allergen challenge in the CEMALB. If the subject has wheezing on physical examination the morning after the inhaled allergen and/or has an FEV1 that is $\geq$15% from baseline measures (indicating that they are still experiencing a late phase response), they will be given a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. These subjects will be contacted by phone within 24 hours of receiving the dose to assess asthma symptoms and will be evaluated in person within 48-72 hours of the dose to monitor FEV1 and to assess the need for continued therapy.

In addition, a follow-up call will be made 48 hours after challenge (24 hours after the follow-up sputum induction) 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.

If the subject requires albuterol for $\geq$48 hours after the inhaled allergen challenge, they will be a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. These subjects will be contacted by phone within 24 hours of receiving the dose to assess asthma symptoms and will be evaluated in person within 48-72 hours of the dose to monitor FEV1 and to assess the need for continued therapy.

Subjects who are withdrawn for medical concerns related to the study, such as but not limited to prolonged decreased FEV_1 or persistent wheezing, will be followed by study physicians until resolution of these events. As this is a screening protocol, these subjects will not be replaced.

A Data Safety Monitoring Board (DSMB) will be established through the NC TraCS Institute to independently review all research activities outlined for the study protocol.

To ensure correct reporting, with each Subject Binder (in the Case Report Form) we have added a discharge worksheet to capture the above information to ensure availability in real time and to guarantee that we do not overlook a violation of safety criteria.

We will use the NC TraCS DSMB to assess for possible changes to the overall risk of the study using inhaled allergen challenge. The DSMB will have full access to research records and source documents. Data regarding unanticipated problems will be provided to them as it is reported to the IRB and to the FDA. They will review all adverse events, enrollment, and protocol deviations after the first 6 subjects have completed the inhaled

allergen protocol, and then every 6 months thereafter. Any serious adverse events or major protocol deviations will be reported immediately to the DSMB via email, which may lead to an earlier review. They will communicate with the PI and the appropriate NIH Medical Officer regarding any safety issues.

Withdrawal or Termination
Participants will be terminated early from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities including follow up.
2. The participant is "lost to follow up" (i.e., no further follow up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The participant develops a medical condition or is started on a new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality of the data obtained from the study.
5. The participant no longer meets inclusion or exclusion criteria as defined in Section 4.1 and 4.2 or if the participant does not meet the previously defined safety criteria (see Section 3.4.2).
6. The study is terminated for any reason.

7.4. Reporting of AEs and SAEs:
UNC-Chapel Hill Human Research Protection Program Standard Operating Procedures will be followed for reporting adverse events/unanticipated problems to the Biomedical IRB and NC TraCS DSMB. All SAEs will be reported to the CBER of the FDA as well as to the UNC OHRE, DSMB, and the NIH sponsor within 24 hours of recognition of the event, including the filing of an IND safety report. 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, the UNC IRB, and the NC TraCS DSMB.

7.5. 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. Withdrawal will also not impact the subject's status as a UNC student or employee. Subjects may be withdrawn if the study physician feels it is in the best interest of the subject.

7.6 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. Records will be kept for 2 years after completion of data collection. 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.

7.7. 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	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	Assistant Professor of Pediatrics UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology
Co-Investigator	Haibo Zhou, PhD 3104C McGavran-Greenberg Hall The University of North Carolina CB #7420 Chapel Hill, NC 27599	Professor of Biostatistics Director of the Biostatistics Core, CEMALB

Co-Investigator	Terry Noah, MD 450 MacNider – The UNC School of Medicine CB 7217 Chapel Hill, NC 27599-7217	Professor and Senior Vice Chair, UNC Department of Pediatrics Division Chief, UNC Pediatric Pulmonology Investigator
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 undergo challenge procedures at the Clinical and Translational Research Center (CTRC) at the University of North Carolina in Chapel Hill, North Carolina, 27599-7310. Additional subject visits and test procedures will be performed at the University of North Carolina Center for Environmental Medicine, Asthma and Lung Biology (CEMALB). All necessary clinical research equipment, medical equipment, and laboratory equipment is located at the CTRC and within the CEMALB.

Institutional Review Board. This study has been submitted to the following IRB: Office of Human Research Ethics 720 Martin Luther King, Jr. Blvd.
Bldg# 385, Second Floor Chapel Hill, NC 27599-7097. This study has received IRB approval, which will be maintained throughout the life of the study.