**SUPPLEMENTAL MATERIAL**

Diesel Exhaust Augments Allergen-Induced Lower Airway Inflammation in Allergic Individuals: A Controlled Human Exposure Study

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**METHODS**

Subjects deemed appropriate by primary screening were invited for a secondary screening where blood was obtained for genotyping and baseline lung function (FEV1), airway responsiveness to methacholine (PC20) was determined and skin prick-positivity was assessed. Sensitization to birch, Pacific grasses and house dust mite (Dermatophagoides pteronyssinus group 1) was tested by skin prick, using standardized extracts (medical grade; Hollister-Stier, Spokane, WA) in a 50% vol/vol glycerin diluent solution. A wheal ≥3 mm to at least one of those allergens was required for inclusion in the study. For subjects with a positive wheal to more than one of the three allergens, the test allergen was chosen based on the season (grass or birch were used in wheal-positive subjects when the duration of the study period for that given subject would be outside the given pollen season; otherwise, dust mite was used). Subjects withheld long-acting β2-agonists for 48 hours, short-acting β2-agonists for 6 hours, long-acting anti-histamines for 14 days, non-steroidal anti-inflammatories and aspirin for 7 days, and short-acting anti-histamines for 3 days prior to skin test and SAC (described below). A series of 1:10 dilutions of the test allergen were used to determine the lowest skin prick dose needed to elicit a 3-mm wheal, based on the strong correlation between the concentration of allergen leading to skin test positivity and that prompting airway responsiveness [1]. In those whose test allergen was birch, we required that they avoid apple, pear, sweet cherry, peach, plum, apricot, almond, celery, carrot, potato, hazelnut, mango, and chili pepper to minimize concerns for oral allergy syndrome.

Recruitment goal was 18 subjects based on a power calculation performed using two-sided paired t-tests with an alpha level of 0.05 and power of 90% based on data in Table 3 of Gilliland *et al* [2], which was performed in the nose but otherwise closely reflected the goals of our analysis. A validated common cold questionnaire [3] was used to confirm that participants were free of viral infection for at least four weeks before each testing session. One hour following each exposure to DE or FA, bronchoscopy was performed to deliver a segmental allergen challenge (SAC 5 mL solution of allergen extract in a concentration 10-fold lower than that minimal dose producing a positive wheal) in a right middle lobe (RML) segment or lingular segment (whether RML or lingula was randomized). The contralateral side (RML or lingula) received a 5 mL diluent control. The locations of the segments used for instillation were carefully recorded by photo to ensure the same segments were sampled at the 2nd bronchoscopy. DE exposure preceded allergen instillation to avoid the possibility that starting with segmental allergen would lead to acute segmental bronchoconstriction and thus decreased deposition of diesel particulate matter within that segment. 48 hours after allergen challenge, bronchial wash (BW; the return from the first 40 cc [20 + 20] instilled saline, which averaged 17.0 mL) and bronchoalveolar lavage (BAL; the return from the following 100 cc [50 + 50] instilled saline, which averaged 54 mL) were obtained in the same segments (allergen- and saline-exposed), for laboratory analysis. The side (RML or lingula) or allergen and saline installations was reversed in the post-washout bronchoscopy and sampling (“Bronch 3” and “Bronch 4” in Figure 2) and different segments were used (for example, if RML medial segment was used in Bronchs 1 and 2, RML lateral segment was used in Bronchs 3 and 4). Spirometry was followed at intervals (1, 2, 3, 4 and 24 hours after exposure) but these data were intended primarily for safety rather than data analysis and are not otherwise presented here.

Given our hypothesis, we considered a four-level ordered factor and fit orthogonal polynomial contrasts. We proceeded with “ordinal effect” analysis only if diesel or allergen effect was p<0.1. Accordingly, ordinal effect results are NA (not applicable) in Tables III-V when diesel and allergen p-value were both greater than 0.1. Orthogonal polynomial contrasts allowed us to test the factor for polynomial patterns in the group means (linear, quadratic and cubic). If the linear pattern was not significant, then we concluded that there was no evidence supporting the hypothesized order, and the ordinal effect was reported as non-significant. If the linear pattern was significant while the quadratic and cubic patterns were not, then there was no evidence against the hypothesized order and we could adequately represent the factor with a line; such cases are considered to have an ordinal effect and the linear p-value is reported.

**Table E1. Markers and flurophores used in flow cytometry for T-cell subsets, eosinophil, and dendritic cell characterization and activation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fluorophore** | **T-cells** | **Tregs** | **Eosinophils** | **DCs** |
| FITC | CD4 | CD4 | CD16 | Lin-1 (CD3, CD14, CD16, CD19, CD20, CD56) |
| PE | CD19 | FoxP3 | CD9 |  |
| PerCP-Cy5.5 | CD3 | CD3 |  | CD123 |
| PE-Cy7 | CD69 | CD69 | HLA-DR | HLA-DR |
| APC |  | CD25 | CCR3 | CD86 |
| APC-Cy7 | CD45 | CD45 | CD45 | CD45 |
| V450 | CD8 | CD45RA | CD69 | CD11c |
|  | T-cells were identified as CD3+. Cytotoxic T-cells were CD8-positive and CD4-negative. T-helper cells were CD4-positive and CD8-negative. | CD3-positive, CD4-positive and FoxP3-high | CD45+ leukocytes that are HLA-DR-low, CD9-high, CD16-intermediate | Lin-1-negative, HLA-DR-positive leukocytes |
|  | CD69 served as the activation marker for all panels except dendritic cells in which CD86 was used. | | | |

**References:**

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