205 Diesel Exhaust Alters Nasal Innate Immune Mechanisms in Allergic Rhinitics

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RATIONALE: We have previously demonstrated that diesel exhaust (DE) exposure enhances virally-induced exacerbation of allergic airway inflammation. We have also shown that natural killer (NK) cells are important immune cells present in nasal lavages, yet the role of NK cells and innate immunity in the inflammatory effects of DE are not known.

METHODS: We conducted a double-blind, randomized, placebo-controlled study of nasal responses to viral infection in the setting of diesel exhaust exposure. 22 human subjects with allergic rhinitis were randomized to clean air or DE (100 μg/m^3) exposure and subsequently nasally inoculated with live attenuated influenza vaccine (LAIV). Nasal lavage was performed prior to inoculation/exposure (day 0) and on days 1, 2, and 7. Cytokine protein analysis and flow cytometry were performed to evaluate alterations in mediator profiles and NK cell surface markers, respectively.

RESULTS: Compared to baseline, LAIV inoculation increased CCL26 (eotaxin-3) and decreased CCL22 (MDC) levels in DE-exposed subjects compared to air-exposed subjects. CCL26 is the ligand for CX3CR1, which is expressed on CD16+ NK cells, a subset of NK cells known for cytolysis. The DE-exposed group also had a higher percentage of CD16+ NK cells, as well as increased levels of granzyme B in nasal lavages. Other chemokines, such as CCL2, CCL3, CCL13, CCL11, and CCL17, did not significantly differ between the two exposure groups.

CONCLUSIONS: In allergic rhinitics, DE exposure alters markers of nasal innate immunity and NK cell activity following viral exposure. This appears to be particularly true regarding mechanisms of innate immunity associated with exaggerated allergic inflammation.

206 Silica Crystals Cause Cellular Injury in TLR3-Activated Human Bronchial Epthelial Cells

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RATIONALE: Exposure to fine-particulate air pollution has the possibility to cause serious health problems including aggravation of asthma symptoms. Silica crystal (Silica) is the main mineral component of yellow sand dust, and activates the inflammasome to induce IL-1β secretion in macrophages. In this study, we examined the effects of Silica on cultured normal human bronchial epithelial cells (NHBE).

METHODS: NHBE were treated with TNF-α, LPS and Poly:IC in the presence or absence of various concentrations of Silica (20-200 μg/ml). Cell viability was assessed by MTT assay and by measuring release of LDH activity and HMGB1 protein. Cell supernatants were analyzed for IL-1β secretion by ELISA and Western blotting. Activation of caspase-1 was examined by Western blotting.

RESULTS: Poly:IC-, but not TNF-α- nor LPS-activated NHBE, showed significant decrease in MTT activity by simultaneous treatment with Silica in a dose-dependent manner. Coincident with the decrease of cell viability, LDH activity, HMGB1 and IL-1β protein in the supernatants of Poly:IC-activated NHBE were significantly increased by simultaneous treatment with Silica. Furthermore, IL-1β protein detected in the supernatants was found to be the precursor form (31 kDa) but not the mature form (17 kDa) by Western blotting. Caspase-1 in NHBE was not activated by a combination treatment with Poly:IC and Silica treatment.

CONCLUSIONS: Unlike macrophages, Silica did not activate the inflammasome in NHBE. Instead, TLR3-activated NHBE were injured by exposure to Silica, probably through induction of necrosis. Our findings suggest that inhalation of yellow sand dust during viral infection may cause severe epithelial damage.

207 Alternaria Induces Stat-6 Dependent Acute Airway Eosinophilia And Epithelial Fizz1 Expression That Promotes Airway Fibrosis And Epithelial Thickness

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RATIONALE: The fungal allergen, *Alternaria*, is specifically associated with severe asthma, including life-threatening exacerbations. To better understand the acute innate airway response to *Alternaria,* inflammatory and epithelial changes were investigated after naïve mice were exposed to a single airway challenge of *Alternaria*.

METHODS: Naïve WT C57/B6 mice were administered a single intranasal challenge with *Alternaria, Candida,* or *Aspergillus* extracts and BAL/lung analyzed 24 hours later. RNA was extracted from airway epithelial cells after bronchial brushing and processed for gene microarray analysis. Immunofluorescent staining of lung sections was performed. Single cell suspensions from lungs were incubated with rFIZZ1, stained for cell type, and analyzed by FACS. Finally, mice were given rFIZZ1 repetitively and lung sections and BAL cell counts analyzed.

RESULTS: Naïve WT mice developed significant BAL eosinophilia following *Alternaria* challenge when analyzed 24 hours later but not after *Aspergillus* or *Candida* challenges. Gene microarray analysis of airway epithelial cell brushings demonstrated that *Alternaria*-challenged WT mice had an over 20-fold increase in level of expression of "Found in Inflammatory Zone 1" (FIZZ1/Retnla) confirmed by qPCR and immunofluorescence. Epithelial FIZZ1 expression as well as BAL eosinophils were significantly reduced in STAT6-deficient, but not PAR-2-deficient mice. rFIZZ1 displayed binding to $CD45^+CD11c^+$ (macrophages and dendritic cells) as well as collagen-1 producing CD45 negative cells (fibroblasts). Direct administration of recombinant FIZZ1 to naïve WT mice led to airway eosinophilia, peribronchial fibrosis, and increased thickness of the airway epithelium.

CONCLUSION: *Alternaria* induces STAT-6 dependent acute airway eosinophila and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness.

208 Chemokines, Soluble Receptors and Mediators of Cord Blood Mononuclear Cells and Atopic Sensitization at 2 Years of Age in At Risk Infants Participating in a Probiotic Supplementation Clinical Trial

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RATIONALE: From a birth cohort of at risk infants (first degree family with atopic disease), we evaluated the influence of intrinsic immunologic risk factors for atopic sensitization at age 2 years to dietary and inhalant allergens.

METHODS: Cord blood samples were collected from 162 subjects of a birth cohort of 253 subjects participating in a double-blind placebo randomized trial on probiotic (Lactobacillus rhamnosus GG and Bifidobacteria longum, birth to 6mths) supplementation. Chemokines, soluble receptors and mediators of lipopolysachharide stimulated cord blood mononuclear cells were analyzed using the Bio-Plex multiplex assay.

RESULTS: At 2 years, 44 subjects developed atopic sensitization and 118 remained non-sensitized. Soluble factors, CXCL6 (Granulocytes chemotactic protein-2), IL-2Ra, and MCSF (Macrophage colony-stimulating factor) were significantly increased in subjects who developed atopic sensitization compared to non-atopics (adj p values: 0.015, 0.005 and 0.008, respectively) after multivariate adjustment for wheeze, birth order, and maternal asthma. A multivariate logistic regression analysis that included all clinical and soluble factors showed that IL-2Ra was the only factor that remained significantly increased (OR 3.3 ; p= 0.002) . Probiotic supplementation did not affect the outcome of atopic sensitization in this study.

CONCLUSION: In infants at genetic risk of atopy, an intrinsic hyperresponsive profile of soluble inflammatory factors is associated with atopic sensitization at age 2 years.