Air Pollution Particle Effects on Human Antimycobacterial Immunity

Megan Rockafellow[1]; Srijata Sarkar[1]; Martha Torres[2]; Alvaro Osornio Vargas[3]; Jim Zhang[4] and Stephan Schwander[1]

[1]*UMDNJ School of Public Health, Department for Environmental and Occupational Health & Center for Global Public Health, Piscataway, N.J.,*

[2]*Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico,* [3] *University of Alberta Edmonton, Canada,* [4]*University of Southern California, USA*

Background

Twenty-five percent of the global disease burden are related to environmental factors.

There is increasing evidence that air pollution increases the rate of respiratory infections worldwide.

Indoor air pollution and cigarette smoking have been shown to be major risk factors for the development of tuberculosis (TB). Epidemiological evidence of significantly increased risk of developing TB after exposure to silica, indoor pollution or cigarette smoking suggests that air pollutants are potentially detrimental to antimicrobial immune effector functions

With a RO1 grant from NIEHS we will study the effects of urban air pollution particulate matter ($PM_{2.5}$) on innate and adaptive human lung immunity against *Mycobacterium tuberculosis* the bacteria that cause TB in Mexico.

$PM_{2.5}$ will be collected in Mexico City and healthy study subjects from Iztapalapa, a municipality in Mexico City known for its high prevalence of TB cases and high air pollution levels, be studied.

We will assess antimycobacterial effector functions of bronchoalveolar (lung) cells before and after *in vitro* $PM_{2.5}$ exposure (see Aims 1 and 2).

Results from Preliminary Studies using Diesel Exhaust Particles as Model Air Pollution Particles in our Laboratory

Figure 1.

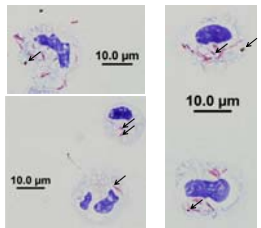


Figure 1. DEP and *M.tb* Uptake in Human Blood Monocytes. Magnetic bead-enriched CD14+ peripheral blood monocytes were stained with Kinyoun acid-fast stain on cytospins following uptake of DEP (black spots indicated with arrows) and *M.tb* (visible as red rods) overnight. DEP was used at a concentration of 10 µg/mL and *M.tb* at a MOI of 10. Five randomly selected monocytes that have incorporated both DEP and *M.tb* are shown. Magnification 1000x.

Figure 2.

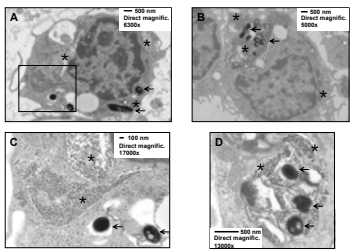


Figure 2. Transmission Electron Microscopy (TEM) of DEP and *M.tb* Uptake in Human Peripheral Blood Monocytes. The micrographs [2A and B: overviews of two independent cells; 2C: detail of 2A (area surrounded by rectangle); 2D, intracellular compartment containing both DEP and three *M.tb*] show uptake of DEP (stars) and *M.tb* (arrows) within the same transects of monocytes, providing evidence of co-localization of DEP and *M.tb* within the cells. Enriched peripheral blood monocytes were cultured with DEP (10 µg/mL) and *M.tb* (MOI 10) for 24 hours and processed as described in Materials and Methods. Scale bars and direct magnifications are included in the figure.

Figure 3.

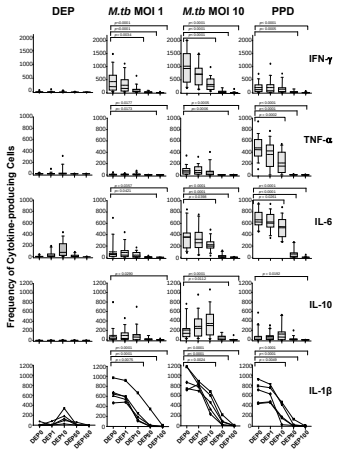


Figure 3. Simultaneous Exposure to DEP Alters Pathogen-induced Cytokine Production. Frequencies of cytokine-producing PBMC (IFN-γ [n=20 subjects], TNF-α [n=18 subjects], IL-6 [n=15 subjects], IL-10 [n=20 subjects, data for DEP 50 µg/mL from n=18 only], and IL-1β [n=5 subjects]) exposed to DEP alone (10 µg/mL), *M.tb* multiplicity of infection (MOI) 1 and MOI 10, and PPD (10 µg/mL) in presence or absence of DEP were measured by ELISPOT assay.

PBMC were stimulated for 4 (TNF-α), 24 (IFN-γ, IL-6, and IL-1β) and 48 (IL-4, IL-10) hours with *M.tb* (MOI 1 and 10) or PPD (10 µg/mL) in presence of indicated amounts of DEP [0 (culture medium control), 1, 10, 50 and 100 µg/mL DEP, respectively].

The Y-axis represents the mean number of cytokine-producing cells in duplicate wells containing 20,000 PBMC for IL-6 and TNF-α, 100,000 PBMC for IL-1β, and 200,000 PBMC for IL-4, IL-10 and IFN-γ. Data are presented in box plots showing from top to bottom: the maximal value (black dot), the 95 (whisker), 75 (mean, dotted), 50 (median), 25 (all as solid horizontal lines), and 5 (whisker) percentiles and the minimum value (black dot). *P*-values are shown on top of box plots where differences in cytokine expression between no DEP and different doses of DEP were regarded to be statistically significant ($p \leq 0.05$).

Hypothesis

Based on our observations in blood cells, we hypothesize that urban ambient $PM_{2.5}$:

(1) impair innate and adaptive antimycobacterial immune effector functions of BACs)

(2) $PM_{2.5}$ alters toll-like receptor (TLR)-mediated *M.tb*-specific cell activation pathways with suppression of several NF-κB and IRF-1-mediated target genes

(3) these alterations lead to altered phagocytosis and decreased *M.tb*-growth-controlling capacity by the BACs *in vitro*;

(4) the induction of cellular toxicity and immune cell functions are modified by baseline exposure of subjects to PM and by their body burden of oxidative stress.

Specific Aims

Aim 1. To assess $PM_{2.5}$-induced cellular toxicity and $PM_{2.5}$ effects on *M.tb*-specific immunity in human BACs.

We will examine the effects of $PM_{2.5}$ on:
(a)cellular apoptosis and necrosis
(b)production of pro- and anti-inflammatory cytokines
(c) Th1, Th2 cytokine and TLR gene expression and signaling
(d) *M.tb*-induced cell activation and Interferon-γ production

Aim 2. To investigate the role of $PM_{2.5}$ in altering phagocytosis and growth control of *M.tb* by human BACs.

We will assess the effects of $PM_{2.5}$ on
(a)phagocytosis of *M.tb* by alveolar macrophages and in stably transfected Chinese Hamster Ovary (CHO) cells expressing scavenger receptor A.
(b)BAC-mediated control of *M.tb* growth using colony forming unit assays.

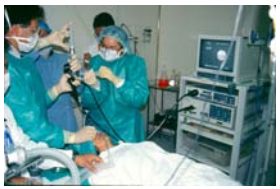
Aim 3. To examine personal *in vivo* PM exposure and its relationship to immune effector functions of BACs.

We will measure and assess
(a) subjects' urinary concentrations of 1-hydroxypyrene, a major metabolite of pyrene as a proxy of combustion-generated pollutants, and 1-aminopyrene, a metabolite of the diesel-exhaust-specific compound 1-nitropyrene, and urinary concentrations of two biomarkers of oxidative stress: 8-hydroxydeoxyguanosine (8-OHdG) marking oxidative DNA damage and malondialdehyde (MDA) marking lipid peroxidation by reactive oxygen species; **(b)** personal exposure using time activity questionnaires and geographical indicators of exposure, (**c**) quantify the particle load in AMs as a long-term exposure measure; and (**d**) statistically examine possible associations between these air pollution exposure measures and the antimycobacterial BAC effector functions and toxicity (Aims 1 & 2).

Study Site

Mexico City is an ideal study site due to its

…try with high TB endemicity



…global trend to urbanization

…ir pollution and TB research

Novelty of Experimental Approach

Our experimental approach expands on current scientific knowledge in several important ways.

First, we will use $PM_{2.5}$ collected from an urban megacity environment that individuals inhale and are exposed to under real-life conditions.

Second, we will study primary human lung cells that are exposed to both $PM_{2.5}$ and *M.tb* aerogenically. Both $PM_{2.5}$ and lung cells of study subjects will be obtained from the same study site within the city.

Third, we will assess healthy individuals who are (a) concurrently aerogenically exposed to *M.tb* in households of individuals with active pulmonary TB (healthy household contacts, HHCs) and (b) healthy control individuals from the same community (CCs, community controls).

Fourth, we will for the first time measure personal $PM_{2.5}$ exposure profiles (urinary biomarkers, time-activity, geographical location, and PM load in AMs) to determine *in vivo* 'baseline' and life-time exposure stratification (high – low) for correlation with antimycobacterial primary lung immune cell effector functions.

Fifth, we will assess the effects of $PM_{2.5}$ on *M.tb*-specific immune cell effector functions (cytokine production, gene expression) and correlate these effects with a *M.tb* growth control assay as a read out of global antimycobacterial immune cell functions.

Significance

PM-induced alterations of innate and adaptive antimycobacterial immune responses may have significant global health implications given the wide geographical scales for both air pollution and *M.tb* infections.

Next Steps

Develop study protocols and consent forms in Spanish and English for IRB submission in Mexico City and at UMDNJ and obtain IRB approval

Develop standard operating procedures for clinical and laboratory research components.

Train participating clinical and lab personnel in Mexico and the US.

Kick off study in 2012

Collaboration Opportunities

If you are interested in opportunities to work in the context of this grant either in the US (UMDNJ School of Public Health) or in Mexico City (National Institute for Respiratory Diseases / Instituto Nacional de Enfermedades Respiratorias) please contact

Dr. Stephan Schwander
School of Public Health
683 Hoes Lane West
Piscataway NJ, 08854
schwansk@umdnj.edu
732-235-5405