Final Technical Report

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Center Name: NYU-EPA PM Center: Health Risks of PM Components
Center Director: Morton Lippmann
Title: Role of PM-Associated Transition Metals in Exacerbating Infectious Pneumoniae in Exposed Rats
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Project Period: June 1, 1999–May 31, 2005 (no-cost extension to May 31, 2006)
Period Covered by the Report: June 1, 1999–May 31, 2006
RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project: Previous investigations in this laboratory demonstrated that a single 5 hr inhalation exposure of *Streptococcus pneumoniae*-infected male rats to concentrated ambient $PM_{2.5}$ from New York City (NYC) air [at concentrations approximating or greater than the promulgated 24 hr National Ambient Air Quality Standard (NAAQS) for $PM_{2.5}$ (~65 - ~150 vs. 50 $\mu g/m^3$, respectively)], altered both pulmonary and systemic immunity, as well as exacerbated the infection process, in a time- post-exposure-dependent manner. These New York University (NYU)-PM Center-supported studies were performed to correlate metal content of ambient $PM_{2.5}$ with its *in vivo* immunotoxicity so as to identify and characterize the role of constitutive transition metals for exacerbating ongoing *S. pneumoniae* infections. The central hypothesis of this particular component was that metals (either individually or in combination) associated with inhaled NYC particulates influenced the severity and/or kinetics of pulmonary bacterial clearance induced by concentrated ambient NYC $PM_{2.5}$. By exposing rats previously-infected with *Streptococcus pneumoniae* (i.e., 48 hr prior to PM exposure) to PM-associated soluble metals, at doses representative of those within the original intact parental PM atmosphere, metals that influence the ability of PM to alter host resistance against infectious agents could be defined.

Soluble metals selected for study included zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), and manganese (Mn), which were based upon those immunomodulating metals identified by XRF analyses and atomic absorption spectroscopy from filters collected during the original NYC study. For the first sets of studies, rats were exposed by inhalation (nose-only) to a single metal at a concentration of 100 $\mu g/m^3$. A dose substantially higher than that found on the original PM atmosphere was selected so as to eliminate those metals having no effect on bacterial host resistance.

Three major objectives were originally proposed to test the aforementioned hypothesis:

(1) To determine whether particle size influences PM-induced alterations in the handling (i.e., uptake and/or killing) of an ongoing pulmonary infection with *S. pneumoniae*.

(2) To identify whether the soluble or insoluble portion of a given size fraction of ambient air PM is responsible for exacerbation of an ongoing pneumococcal-associated pneumonia.

(3) To ascertain which transition metals (either individually or in combination) found most active in the previously identified portion of ambient PM play significant roles in exacerbating ongoing pneumococcal-induced pneumonia in PM-exposed hosts.

Unfortunately, due to a number of technical and personnel difficulties encountered throughout the project the originally proposed hypothesis and specific aims were modified shortly after project initiation. The redesigned **working hypothesis** tested the notion that particle solubility, and/or metal constituents of PM play a critical role in mediating PM-associated pneumonia-related morbidity/mortality in exposed individuals. In this case, the role of metals (alone and in combination with each other), as well as the individual physico-chemical attributes of the metals that influence the ability of PM to alter host resistance against infectious agents, could be defined. The major infection endpoint investigated in these studies remained the same as previously proposed. Specific Aim 1 was deleted and the second aim was modified such that composition and solubility of metal particles were determined from within a single PM size range. Since NYC studies could not be performed due to the lack of a "workable" concentrator at that time, Aim 3 was also deleted and the role of solubility and the identification/quantitation of metal constituents in concentrated PM were determined from NYC filter samples collected previously for the Health Effects Institute (HEI) study.

Summary of Findings:

Technical Aspects

Soluble metals selected for study including Zn, Fe, Cu, Ni, and Mn were based upon those immunomodulating metals identified from filters collected during the original NYC study. In the first sets of studies, rats were exposed to a single metal at a concentration of 100 $\mu g/m^3$. A dose substantially higher than that found on the original PM atmosphere was selected so as to eliminate those metals having no effect on bacterial host resistance. Iron, Zn, and Ni proved most biologically active in this capacity. In addition to host resistance, a number of immune parameters important for resistance of the host against infectious bacterial pathogens were also evaluated. These included: pulmonary histology; lung cell numbers and profiles; lavageable lactate dehydrogenase activity; total protein levels and cytokines; macrophage-mediated production of reactive oxygen species; splenic lymphocyte proliferation; and circulating blood cell profiles. Even at this relatively high metal dose, inhalation of either Cu or Mn had little or no effects on these particular immune parameters. Similar to that observed for host resistance, Zn, Fe, and Ni, had the greatest effects on these biological endpoints. Based upon these results, only these three metals were evaluated at more relevant concentrations. In this case, only Fe and Ni altered host resistance at a 10-fold lower concentration (i.e., 10 $\mu g/m^3$); Fe compromised pulmonary bacterial clearance by about 60, while exposure to 10 μg Ni/m^3 actually enhanced clearance by ~30%. Given that: 1) PM-associated metals don't exist in isolation; 2) the biological effects of exposure to PM likely depend upon responses to metals in combination; and 3) exposure to pollutant combinations often results in responses different from those seen

following inhalation of individual materials, mixture studies were performed to examine the interactive toxicity of Zn, Ni, and Fe on anti-bacterial defense mechanisms and the "handling" of ongoing pneumococcal infections. At an equimolar metal concentration of 50 μg/m^3, rats were exposed simultaneously to Cu plus Ni, Zn plus Ni, or Fe plus Mn. Both Cu and Mn significantly antagonized the pulmonary toxicity of Ni and Fe, respectively. On the other hand, exposure to Zn acted to reverse the "beneficial" effects of Ni alone on pulmonary bacterial clearance; simultaneous exposure of Zn and Ni reduced clearance of Streptococcus by about 30%.

Conclusions

These studies demonstrated that even an acute (5 hr) exposure to PM-associated metals including soluble Fe, Zn, and Ni act to exacerbate an ongoing *S. pneumoniae* infection in particle-exposed rats. Moreover, these same metals in combination can produce responses different from those seen following inhalation of the individual metals alone. This study has provided necessary information as to the particular PM constituents/metal interactions responsible for the observed effects upon host immunocompetence. Taken together, results of these investigations provide biological plausibility for the role of certain PM-associated transition metals to worsen the outcome of an ongoing pulmonary infection.

Supplemental Keywords: NA

Relevant Web Sites: http://www.med.nyu.edu/environmental/
http://es.epa.gov/ncer/science/pm/centers.html