INTRODUCTION

BACKGROUND

- Extensive WW2 and Cold War uranium mining on Navajo Nation (NN) lands left more than 1,100 unremediated uranium mines and waste sites
- Long-held concern of Navajo community members that exposures to environmental uranium (EU) contribute to poor health outcomes among tribal members
- Community concerns were: an increased risk and prevalence of autoimmune diseases among Navajos and more severe infections among children and the elderly
- Possible exposure to legacy mine wastes through multiple exposure routes
  - Contaminated water consumption as 35% of community members has no access to safe drinking water
  - Dust inhalation that introduces metal mixtures to the lung and its immune cells
  - Radiation exposure (probably less likely route)

We have two community-based participatory research projects examining environmental metal and metal mixture exposures and their possible immune system responses.

1. Diné Network for Environmental Health (DiNEH) project initiated in 2001 to address community concerns on mining era and legacy uranium exposures.
   - Total of 1,304 participants with detailed health survey information and immune function studies on subset of DiNEH participants (n > 268) were carried out in 2010-2011 and ongoing.
2. Navajo Birth Cohort Study (NBCS) investigates 1,500 pregnant women and their families living near uranium sites and left more than 1,100 unremediated uranium mines and waste sites.
   - Immune function studies on subset of DiNEH participants (n = 268) were carried out in 2010-2011.
   - Goal of that prospective birth cohort epidemiological study is to examine environmental metal and metal mixture exposures and their complex metal mixtures and body burden.
   - Radiation exposure (probably less likely route)

METHODS

- Blood samples collected from 268 DiNEH study participants representing 20 Navajo chapters of the Eastern Agency (New Mexico side of NN)
- Serum was separated on site at collection and samples were kept at -80°C for appropriate storage
- High sensitivity quantification of 13 important serum cytokines of xMAP bead-based technology (Millipore Inc., Miliplex magnetic bead assay) and Magpix detection system was used to generate inflammatory cytokine results and establish U concentrations in pg/mL, LOD: 0.13 pg/mL.
- High and low concentration of positive controls were used in each run of cytokine measurements for quality control
- Stored urine samples recently were pulled and measured (unfiltered urine, Ni, Zn, Cu, and Cd) and for total arsenic biomonitoring using ICP-MS technique (see Acknowledgement section) on all collection, no budget was available for biomonitoring.
- Statistical approach:
  - Multivariate linear regression modeling was used to examine association between distance from legacy uranium mine waste features, mining era U exposure, traditional risk factors (age, gender), most recently accomplished urine metal and metalloid exposures and immune response biomarkers for DiNEH participants (Table 2).
- Summary statistics available for NBCS samples (Table 1.)
- Previously presented information on lymphocytes subpopulation changes and autoantibody prevalence (7th Metal and Carcinogenesis Conference, 2012 Albuquerque, NM).
- New information on autoimmune molecular markers can be found on D. MacKenzie et al. poster #27.

RESULTS

INFLAMMATORY CONDITIONS AND CYTOKINE MEASUREMENTS

The per cent of variance accounted for these above statistical models, IL-1β and IL-4 are the most sensitive cytokines, both significantly decreased by urine U, increased by Ni, and responsive to different metal mixtures.

Biomonitoring results of urine U, 69.4% of adults and 87% of babies had urine U concentrations above the NAIHANES 50th level (marked as lower linear line on graphs).

To be able to establish effect sizes for U and humoral immune response evaluations, and to investigate further DiNEH study findings (Figure 2-4), we selected mother-baby pairs (at delivery time point samples N=134) with low (below LOD) and high (above 50th percentile) urine U concentrations for multiple cytokine assays.

In addition, we included a smaller number of father serum samples and individuals who had elevated ANA results (please see Poster #27) regardless of their U exposure.

RESULTS (cont.)

STATIONARY MODELING RESULTS


table

CONCLUSIONS

- Indications of EU-related altered immune response: DiNEH study individuals in higher EU variable had significantly lower cytokines production, while we detected inflammation in DiNEH study population with lower EU variable (Figure 2-4). This finding warrants further investigation and in vitro-based research approach.
- The percent of variance accounted for these above statistical models, IL-1β and IL-4 are the most sensitive cytokines, both significantly decreased by urine U, increased by Ni, and responsive to different metal mixtures.
- Based on descriptive statistical information of the NBCS cytokine measurements, baby samples had significantly more non-selectable levels compared to adults. However, many of the detectable levels were several fold below detectable concentrations (4%, IL-4, IL-6, IL-8 and TNFα). Several of these cytokines reported in literature as increased levels normal and necessary at birth to prime healthy immune response and stimulation of innate immunity.
- FUTURE DIRECTIONS
  - Implant concentration ranges of biomonitoring information of metal exposure in vitro cell modeling to confirm immune alteration findings.

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