Environmental Metal Exposures and Immune System Responses — Results from two community-based participatory research studies on the Navajo Nation

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INTRODUCTION

BACKGROUND

- Extensive WWII 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 inflections 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.
   - 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 (14-45 yrs) enrolling at 6 clinical IHS sites; follows and evaluates their newborn babies up to 12-month of age.
   - Goal of that prospective birth cohort epidemiological study is to elucidate associations related to environmental uranium, other metal exposures confirmed by biomonitoring from blood, serum and urine samples.
   - Focuses on birth outcomes and development delays as significant child health outcomes of U exposures.

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^{o}C for appropriate storage
- High sensitivity quantification of 13 important serum cytokines of xMAP bead-based technology (Millipore Inc., Milliplex magnetic bead assay) and Magpix detection system was used to generate inflammatory cytokine results and establish Th1/Th2 balances (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, N=200 for 4 metals (U, Ni, V, Cu) and for total arsenic biomonitoring using ICP-MS technique (see Acknowledgement section) At 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

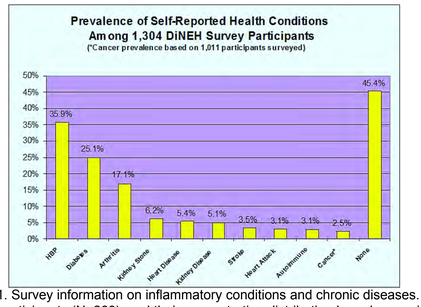


Figure 1. Survey information on inflammatory conditions and chronic diseases. participants (N=268) and their concentration distribution based on environmental exposure measures and proximity to uranium mining waste (EU)

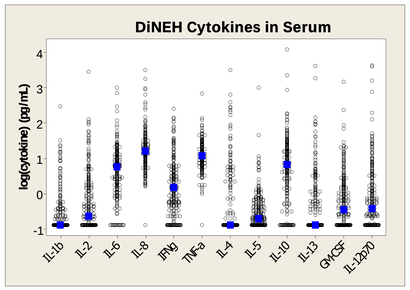
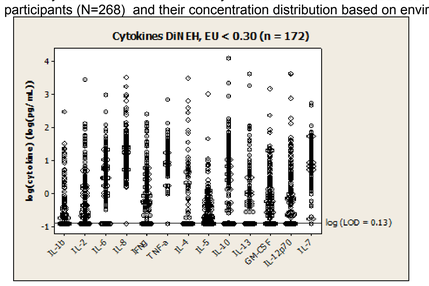
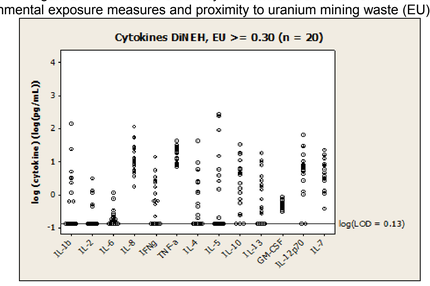


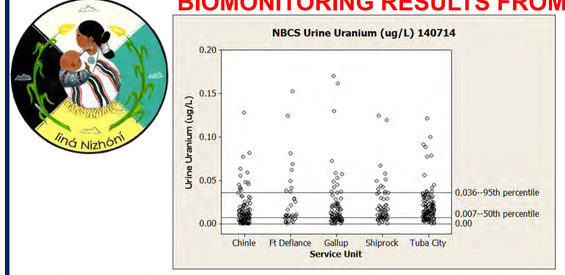
Figure 2-4. 13 human serum cytokine measurements from the DiNEH project

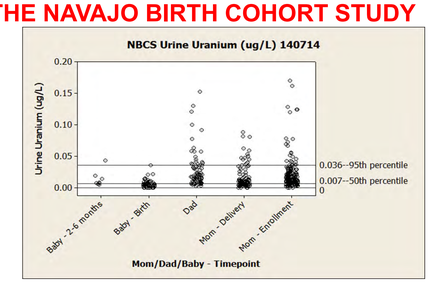




Close proximity to uranium waste resulted in overall lower production of cytokines, whereas people living greater distance from U waste sites had increased production of inflammatory cytokines detected. DiNEH project had no resources for detailed biomonitoring to characterize complex metal mixtures and body burden. To confirm the observed findings of immunosuppresive effects of U exposures on cytokine production among Navajo community members, we used immune system examinations in our new population-based prospective epidemiological study , the Navajo Birth Cohort Study. In that study CDC/ATSDR provides comprehensive metal biomonitoring data generated by CDC/NCEH/DLS/Inorganic and Radiation Analytical Toxicology Branch Laboratory, Atlanta, Georgia.

BIOMONITORING RESULTS FROM THE NAVAJO BIRTH COHORT STUDY





Based on biomonitoring results of urine U, 69.4% of adults and 87% of babies had urine U concentrations above the NHANES 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=34) with low (below LOD) and high (above 50th percentile) urine U concentrations for multiplexing 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.

Cytokines	Mothers (N=34)			Babies (N=34)			Fathers (N=8)		
	Mean cc. (pg/mL)	SD (pg/mL)	Non-detects	Mean cc. (pg/mL)	SD (pg/mL)	Non-detects	Mean cc. (pg/mL)	SD (pg/mL)	Non-detects
GM-CSF	504.21	993.86	6	56.90	99.5	29	695.18	1178.76	1
INF-γ	5.19	4.16	1	32.66	55.78	29	4.07	3.67	0
IL-1β	1.47	1.37	5	1.97	3.06	23	2.67	1.77	3
IL-2	6.57	14.47	4	3.57	6.89	29	6.49	8.44	1
IL-4	5.56	4.55	12	83.57	139.04	30	13.53	10.56	4
IL-5	1.71	1.07	6	1.70	0.96	28	2.41	3.01	1
IL-6	8.07	10.23	0	23.73	58.60	3	2.61	1.67	0
IL-7	10.39	7.84	0	6.18	6.15	19	11.78	4.23	0
IL-8	8.51	5.89	0	64.23	135.39	0	9.95	4.92	0
IL-10	10.98	26.98	2	18.41	42.05	1	10.24	13.85	1
IL-12	3.96	4.83	3	5.81	9.95	27	5.84	5.58	1
IL-13	5.38	6.49	5	9.40	15.12	29	3.28	2.43	0
TNF-α	8.02	5.68	0	26.78	15.60	0	11.04	4.44	0

RESULTS (cont.)

STATISTICAL MODELING RESULTS

Cytokine	Significant non-metal predictors	Urine metal predictors	Significant Interactions	R^2
GM-CSF	**Gender (+)** **Age (+)**	**Ni (+)** **As (-)**	**Gender x As (-)** **Age x V (+)** **Age x Ni (-)** **Age x As (+)** **V x As (-)**	**0.21**
IL-13	BMI (-) M (Mining Era Exposures) (+)	U (+)	Gender x Age (+) Gender x As (-) Age x M (-) Age x V (+) V x As (-)	0.49
IL-1β	Age (+) Gender (-) BMI (+) M (Mining Era Exposures) (+)	U (-) Ni (+)	Age x Gender (+) Age x BMI (-) Age x V (+) Age x Ni (-) U x As (+) V x As (-) U x V (+)	0.93
IL-2	Gender (+) Age (+) M (Mining Era Exposure) (-)	U (-) Ni (+)	Gender x U (+) Gender x Ni (-) Age x V (+) Age x Ni (-)	0.22
IL-4	Gender (+) E (Legacy Waste Exposure) (-)	U (-) Ni (+)	Gender x BMI (-) Gender x E (+) Gender x V (+) Gender x Ni (-) Age x U (+) Age x Ni (-) U x As (+) V x As (-) U x V (+)	0.78
IL-5	BMI (-) M (Mining Era Exposures) (+)	Ni (-) As (+)	Gender x M (-) Gender x Ni (+) Gender x As (-) U x As (+) V x As (-)	0.21
IL-17		V (-)	U x As (+) V x As (+) U x V (-)	0.50

DISCUSSION

CONCLUSIONS

- Indications of EU-related altered immune response: DiNEH study individuals with higher EU variable had significantly lower cytokine 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 by these above statistical models, IL-1-β and IL-4 are the most sensitive cytokines, both significantly decreased by urine U, increased by Ni, and responsive interactions among U, As and V.
- Based on descriptive statistical information of the NBCS cytokine measurements, baby samples had significantly more non-detectable levels compared to adults. However, many of the detectable levels were several times of the adult concentrations (IFN-γ, IL-4, IL-6, IL-8 and TNF-α). 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
- Implement concentration ranges of biomonitoring information of metal exposure to *in vitro* cell modeling to confirm immune alteration findings.
- Statistical modeling on more NBCS sera in association with detailed biological monitoring of metals.

ACKNOWLEDGEMENTS

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