Immune Response Alterations Among Participants in Diné Network for Environmental Health (DiNEH) Project

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October 2014
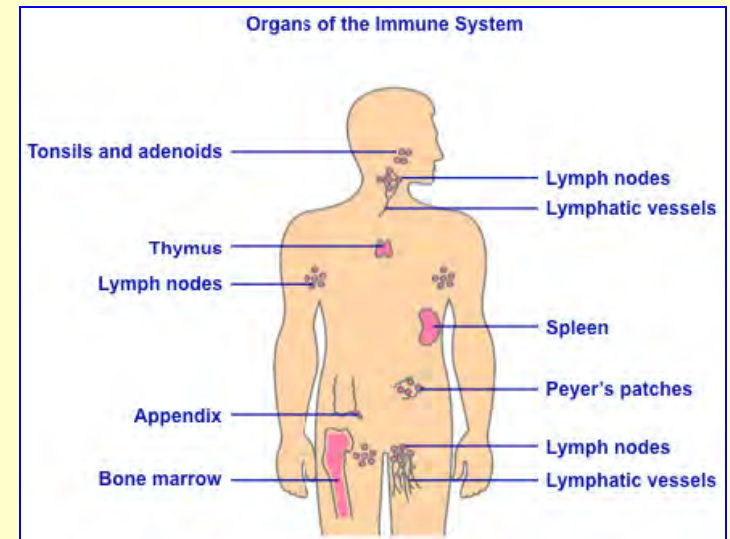

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Immune system and autoimmunity

➢ Immune system — human body's defense system

➢ Processes work to ensure survival of the individual and reinstate homeostatic conditions after infection

➢ Early markers produced by immune cells, showing alterations in immune cell distribution and activity

➢ Autoimmune diseases — conditions resulted in disturbance of healthy immune function, hyperactivity and overproduction of immune activation against the body's own tissues, organs

➢ Fernald (Ohio) studies – found association between uranium exposures and incidence of lupus, a chronic inflammatory disease

Source: http://www.rheumatology.org/About/Newsroom/PressReleases/Uranium_Exposure_Linked_to_High_Lupus_Rates_in_Community_Living_Near_a_Former_Refinery/

Organs of the Immune System

Tonsils and adenoids

Lymph nodes

Lymphatic vessels

Thymus

Lymph nodes

Spleen

Peyer's patches

Appendix

Lymph nodes

Bone marrow

Lymphatic vessels

Examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, systemic lupus erythematous, psoriasis, scleroderma, and autoimmune thyroid diseases.

Source: http://thyroid.about.com/od/thyroidbasicsthyroid101/l/blexamplesa.htm

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Studies of human immune response among DiNEH participants: methods

*Dr. Erdei supervises processing of blood samples.*

- ➢ Characterized immune cells and subpopulations
- ➢ Cellular changes examined for 69 DiNEH participants (out of 268 who provided biological samples)
- ➢ Measured production of certain proteins called cytokines
  - ▪ All 268 participants examined for 13 important human cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, TNF-α, INF-γ, GM-CSF
- ➢ Describe and establish prevalence of autoantibody (autoAbs) production against common cellular antigens (ANA positivity + lupus and connective tissue-specific autoAbs)
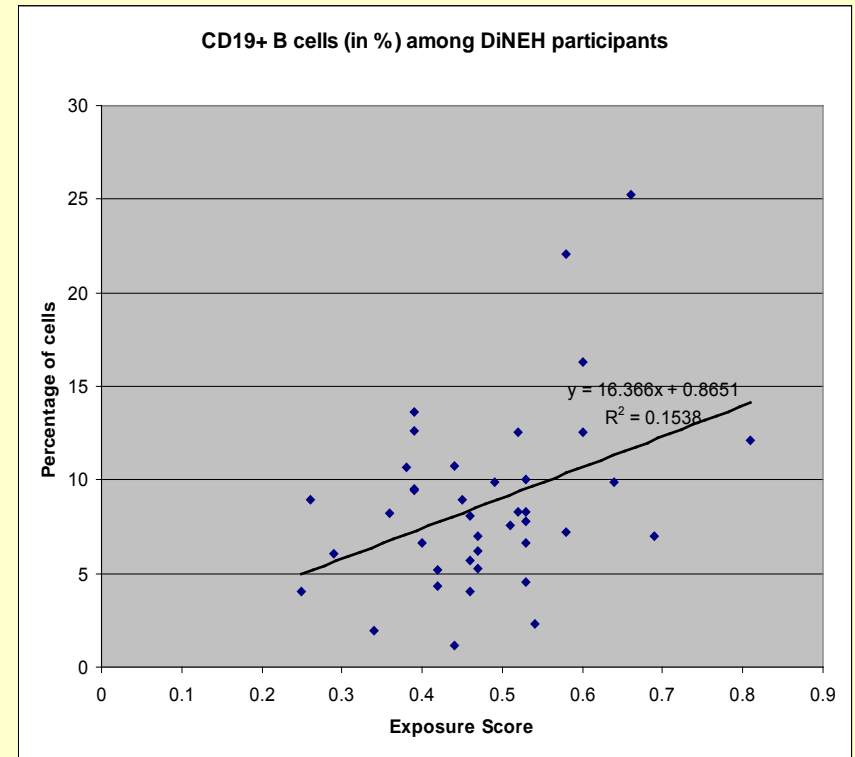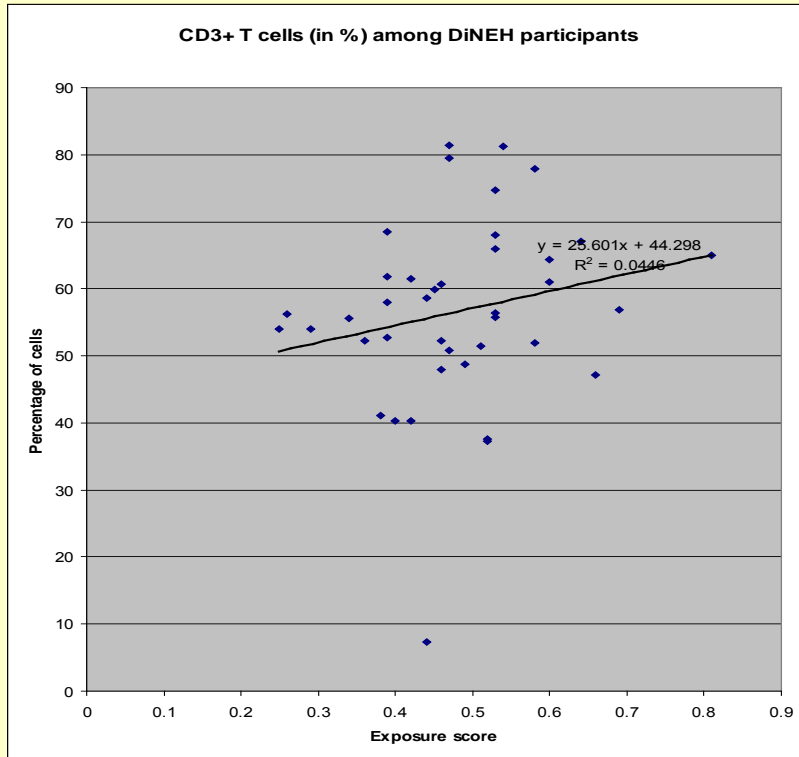
Flow cytometry* measurements

➤ Lymphocyte subpopulations from whole blood samples can be simultaneously measured; no cell separation, no lysis needed

➤ Used Becton Dickinson Simultest IMK Plus lymphocyte kit

➤ 6 cell populations were measured:

- T cells (CD3+), T helpers (CD4+), T suppressors (CD8+);

- B cells (CD19+)

- HLA-DR+ cell activation in T cells

- B cells and other cell types HLA-DR+ expression

- NK (natural killer) cells (CD3-/CD16+/CD56+)
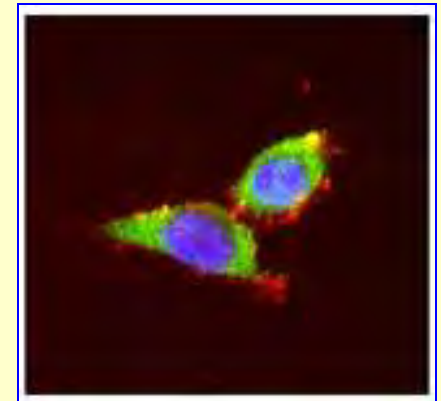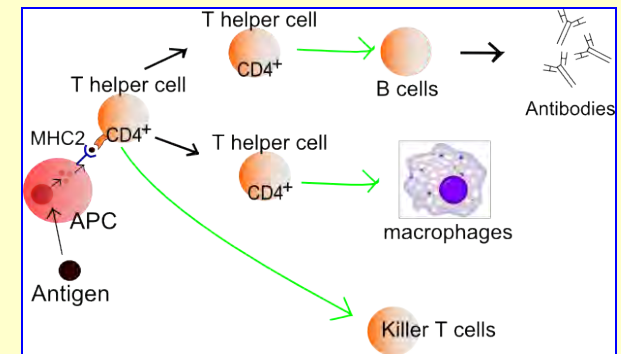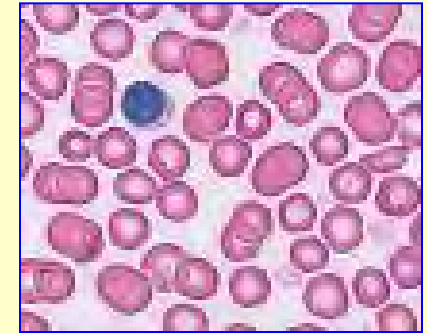
Flow cytometry is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.

Immune impairment associated with U exposure

CD3+ T cells (in %) among DiNEH participants

$y = 25.601x + 44.298$
$R^2 = 0.0446$

Percentage of cells / Exposure score

CD19+ B cells (in %) among DiNEH participants

$y = 16.366x + 0.8651$
$R^2 = 0.1538$

Percentage of cells / Exposure Score

➤ Detected lower mean value for T cells among Navajos compared with Caucasians

➤ Observed increased percentage of activated T cells associated with living in close proximity to uranium mine waste and having activities bringing them in contact with waste

➤ Detected lower mean value for B cells among Navajos compared with Caucasians

➤ B cells showed similar response to U exposure as T cells (left)

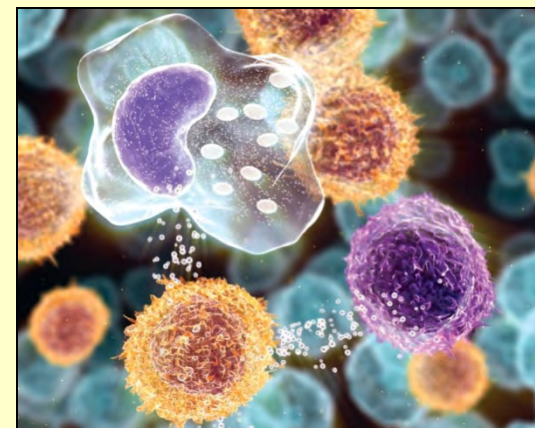➤ HOWEVER →

Altered immune response related with U exposure

➢ Decreased percentage of activated B cells associated with living in close proximity to uranium mine wastes (figure on right)

➢ ***In healthy immune system, T cells and B cells act together, changes expected to happen in the same direction***

➢ This *decoupling* of T cell and B cell activities suggests altered immune response among this subset of participants *exposed to uranium wastes*

➢ Can lead to lower production of protective antibodies and altered immune responses (B cell point of view)

Activated B cells and other antigen presenting cells (in %) among DiNEH participants
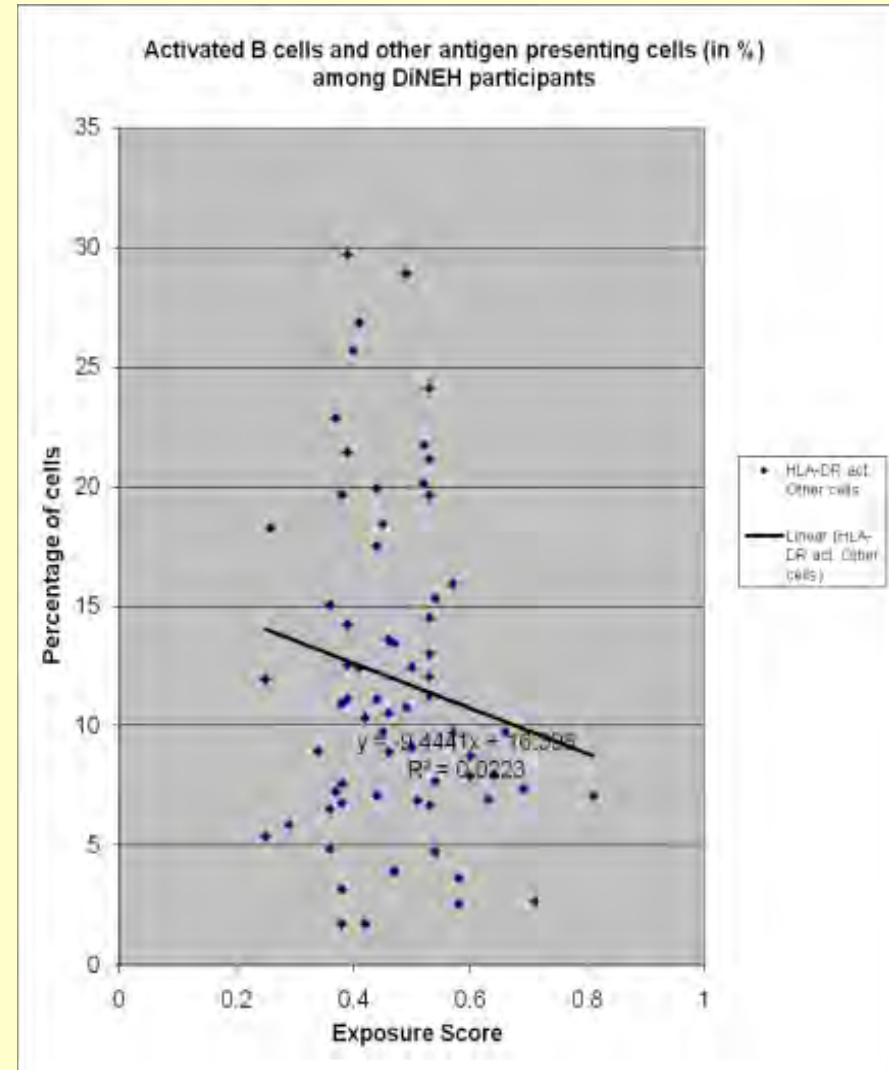
Serum cytokine measurements

- *Cytokines* defined: Diverse group of non-antibody proteins that act as mediators between cells and regulators of immune processes

  http://pathmicro.med.sc.edu/mobile/m.immuno-13.htm

- Applied xMAP multiplexing technology (50 different proteins can be measured all at once), magnetic bead-based detection, high sensitivity assay, detects proteins circulated at very low concentration (pg/ml) in the blood stream

- Detection of 13 human serum cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, TNF-α, and GM-CSF)

- Uses only 25 μl of serum sample/participant, not too much to collect, community-based research!

- Complex task: *Cytokine production indicative of the active T cell populations, Th1 and Th2 balance, presence of an inflammatory response, that can be associated with exposures to various metals and metal mixtures*

Source: http://multiple-sclerosis-research.blogspot.com/2013/01/research-cytokines-and-progression.html

Environmental uranium (EU) exposure and cytokine production

(a) Cytokines DiNEH, EU < 0.30 (n = 172)

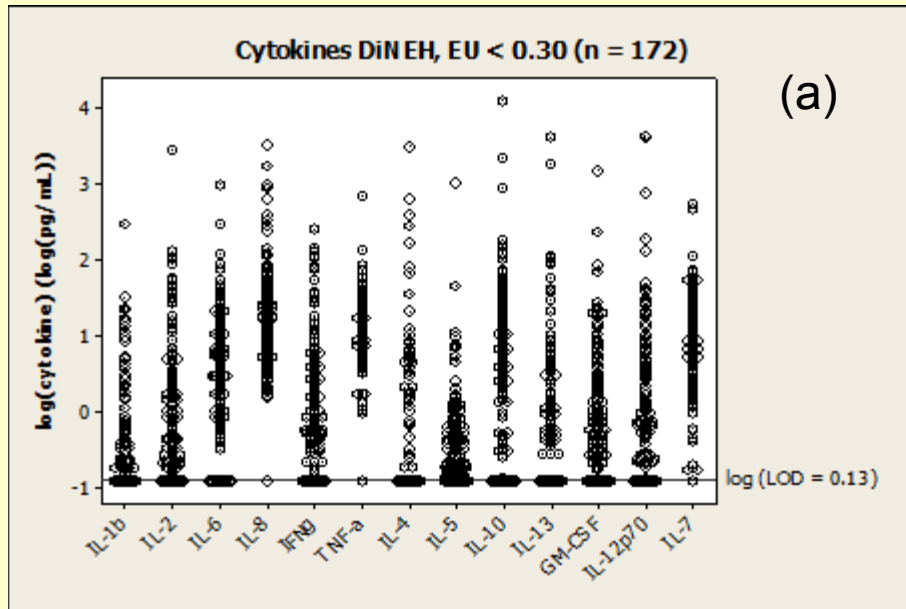(b) Cytokines DiNEH, EU >= 0.30 (n = 20)

Figure (a) shows participants' cytokine values (log scale) with lower EU variable, less contact and activities with mine waste
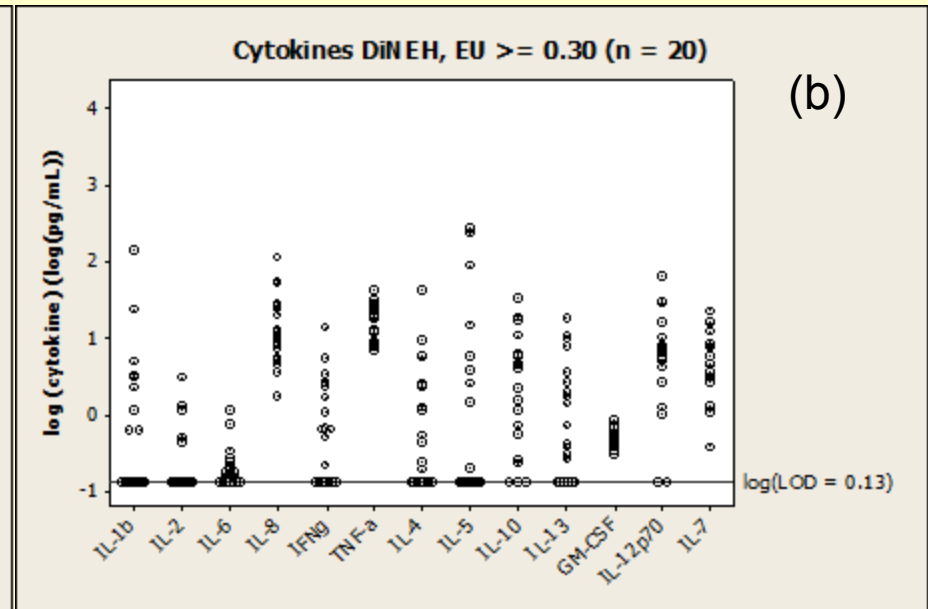
Figure (b) Decreased cytokine concentrations were detected among people living close to abandoned U mines or milling waste and reported more activities that brought them to contact with this waste, which contains mixtures of many metals

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Autoantibody production and detection

<u>243 samples analyzed at IHS LabCorp in Phoenix, AZ (certified clinical diagnostic laboratory):</u>

➤ Blood samples collected in collaboration with NAIHS/CUE-JTH* medical monitoring program

➤ <u>Antinuclear antibody</u> (ANA) testing
- Fluorescence staining and microscopy (traditional method; figure, top right) vs. new faster technique

➤ After positive results obtained, special <u>panel of autoantibodies</u> tested in 119 individuals using flow cytometry-based microbead assay
- Positive response to specific autoantibodies may indicate connective tissue disease, Sjögren's syndrome
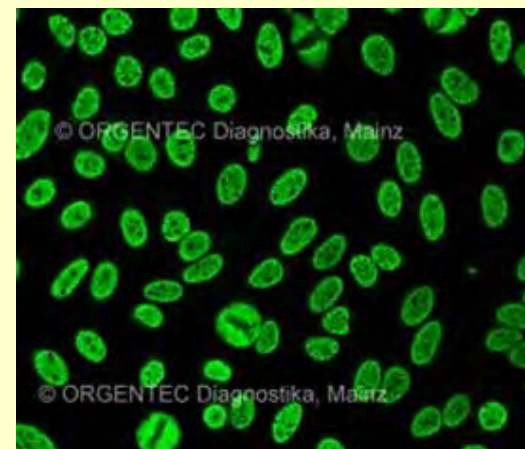
*NAIHS/CUE-JTH = Navajo Area Indian Health Service, Community Uranium Exposure-Journey To Healing program, initiative to provide medical monitoring to U-exposed Navajos with medical needs.
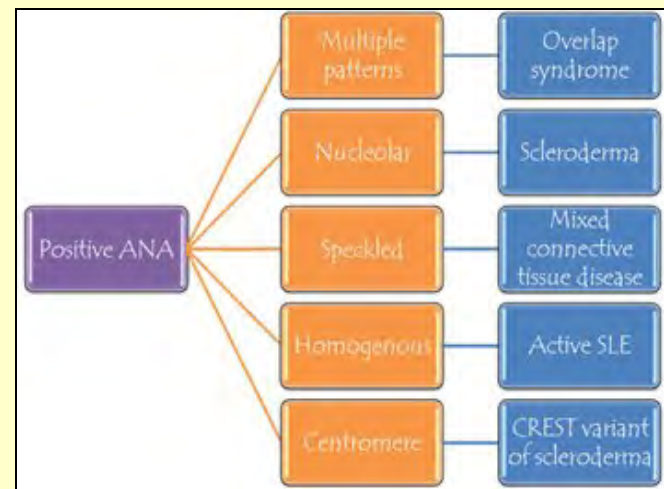
Autoantibody production and detection: results from LabCorp

- ANA positivity among DiNEH participants was 48%, or 3.5 *times higher* than national average (13.8%)
  - ANAs used in diagnosis of some autoimmune disorders
  - ANA positivity is an alternation of the normal, healthy immune response
- Techniques used to detect ANA differ widely between laboratories
- Known that ANA positivity increases with age
- High titer indicates connective tissue disease is likely if clinical findings are present
- False-positive results occur in normal blood donors and in patients with chronic liver disease, neoplasms, or active chronic infections

Special panel of autoantibodies results

- Positive ANA, at least 1 positive special Ab result, was also obtained; SS-A, SS-B positives were the most frequent

Autoantibody production and detection from UNM Lab

<u>At UNM Laboratory</u> (collaboration with Prof. Bob Rubin)

➤ These specific autoantibodies were developed by Dr. Rubin to be able to distinguish environmental insult- induced production vs. genetically-driven autoantibody production

➤ Enzyme-linked immunosorbent assay (picture) was carried out to detect

- <u>anti-denatured DNA</u> — Ab produced against own genetic material, single-stranded, not clinical; linked with cellular death process
- <u>anti-native DNA</u> — Ab against complete genetic material; clinical, lupus diagnosis
- <u>anti-histone</u> — Ab against proteins scaffolding genetic material
- <u>anti-chromatin</u> — Ab against complete protein and DNA structure, nucleus

➤Reasons:

- metal exposures → increased oxidative stress, cell death (apoptosis)
- increased apoptosis leads to increased cellular antigen presentation → increased autoantibody production

Autoantibody production and detection: UNM lab results

➤ Percentages of participants with autoantibodies more than $\pm$2 SD above the mean optical density established for background of the ELISA test
- denatured DNA: 16.1 %
- native DNA: 4.5%
- histone-specific: 1.9%
- chromatin antigens: 3.7%

➤ Clinical importance:
- *Anti-native DNA* should be 0
- DNA positive individuals will be screened using their medical records

➤ *Chromatin specific Ab* is extremely sensitive for lupus

Summary and Discussion of Findings and Conclusions

➢ DiNEH participants have 3.5 times higher rate of antinuclear antibodies (ANA) than the U.S. population, indicating that a substantial portion of the Navajo population has altered immune responses

- New experiments using serum samples from participants in the Navajo Birth Cohort Study demonstrate that younger Navajos (mean age: 26 yrs) also produce ANA autoantibody at higher prevalence compared with US national average (20% vs. 13.8%).

➢ Inflammatory conditions are present among individuals with lower exposure scores, but strong suppression of cytokine response was detected among highly exposed DiNEH participants

Future Directions

➤ Need to implement *in vitro* and animal studies to validate findings

➤ Detailed evaluation of cellular activation/inhibition in different subsets of lymphocytes

➤ Will address Th17 and Treg populations and their contributions to autoimmune responses in consecutive grants

➤ Will establish uranium transport biomarkers to understand actual body burden of U and other metals