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In utero arsenic exposure and fetal immune repertoire in a US pregnancy cohort

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Abstract Arsenic has wide-ranging effects on human health and there is evidence that it alters the immune response by influencing CD4+/CD8+ T cell ratios, IL-2 cytokine levels, and the expression of immune-response genes. We investigated the impact of *in utero* environmental arsenic exposure on immune development and function in newborns participating in a pregnancy cohort in New Hampshire, U.S., where arsenic levels have exceeded the current EPA maximum contaminant level of 10 μg/L. Our results showed that maternal urinary arsenic concentrations were inversely related to absolute total CD45RA+ CD4+ cord blood CD69+ T cell counts (N = 116, $p = 0.04$) and positively associated with CD45RA+ CD69− CD294+ cell counts ($p = 0.01$). In placental samples (N = 70), higher *in utero* urinary arsenic concentrations were positively associated with the expression of IL1β ($p = 0.03$). These data provide evidence that relatively low-level arsenic exposure *in utero* may alter the fetal immune system and lead to immune dysregulation.

Abbreviations: Treg, regulatory T cell; APCs, antigen-presenting cells; AQP9, Aquaporin-9; IL1β, Interleukin 1 beta.

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1. Introduction

Arsenic has wide ranging human health effects, including its established role as a human carcinogen and emerging data suggesting that it enhances susceptibility to infection [1–4]. Arsenic has been shown to affect multiple aspects of immune function, such as T cell ratios [5], IL-2 cytokine levels, and the expression of immune-response genes, including those involved in T cell receptor signaling [6–8]. These arsenic-associated changes in lymphocyte subsets and cytokine profiles could impact not only susceptibility to infection, but also risk of atopic disorders.

T cells represent a subset of immune cells that play a key role in fighting infections, in vaccine responses, and in promoting inflammation [9–13]. Once a T cell becomes activated, T cell effector functions can initiate downstream events, such as the synthesis of antibodies by B cells. CD4+ T cells play a role in the etiology and maintenance of immune diseases such as allergies [14]. Different subsets of CD4+ T cells in the peripheral blood can be further identified phenotypically by surface markers as memory (CD45RO+) or naïve (CD45RA+), proliferating (Ki67+) or activated (CD69+), Th1 (IFN-γ containing) or Th2 (CD294+) by standard flow cytometry techniques [10,12]. Another subset of T cells, regulatory T cells (Treg, defined by CD4+ CD25hi CD127lo cells), usually increases in response to inflammatory processes [9,13]. T cells, especially Treg, have been shown to influence pregnancy outcomes [15,16] and are affected by environmental pollutants [11,17].

Gestation is a critical period for immune development [18] and there is evidence that Th2-skewed responses to common environmental allergens occur in newborns [19,20]. Given their limited exposure to antigens, most T cells in cord blood are presumed to be naïve (*i.e.*, CD45RA+) and can become activated during inflammation [15,16]. However, circulating neonatal T lymphocytes are fundamentally different from naïve adult T cells and have characteristics of recent thymic emigrants that are impaired in their acquisition of Th1 function [21]. Additionally, a high proportion of circulating neonatal T cells are concurrently progressing through the cell cycle and have increased susceptibility to apoptosis, as compared to those of adults [22]. While strong experimental evidence demonstrates that arsenic exposure can alter immune function [8,23], few human studies have examined the effects of arsenic on T cells, and in particular, the developing T cell repertoire of the neonate.

The prevalence of atopy and allergic diseases have increased dramatically in recent years [24,25], affecting up to 30% of the population in industrialized countries [12] and causing substantial childhood morbidity, as well as social and medical costs. At the same time, infant infections remain prevalent in the first year of life, even in developed countries [26,27]. We have established a pregnancy cohort comprised of individuals with exposures spanning the dose range of regulatory interest, (*i.e.*, water arsenic 0–100 μg/L, with over 10% of the cohort's well water samples exceeding the US EPA standard of 10 μg/L arsenic in water). As part of this cohort study, we investigated whether neonatal T cells are potential targets of arsenic immunotoxicity by examining changes in immune cell profiles, T cell functionality and gene expression measures.

2. Methods

2.1. Ethics statement

Study protocols and materials were approved by both the Dartmouth Committee for the Protection of Human Subjects and Stanford Administrative Panels on Human Subjects in Medical Research. All subjects provided written informed consent to participate.

2.2. Study subjects

Pregnant women who obtained their prenatal care at clinics in the New Hampshire area, including regions with relatively high water arsenic levels identified by our earlier work [28], were enrolled at 24–28 weeks of gestation beginning in 2009. T cell functions were analyzed for 16 cord blood samples from the extremes of exposure: high arsenic (>5 μg/L arsenic in water, and >5 μg/L total urinary arsenic, n = 8) and low arsenic exposed women (<0.5 μg/L arsenic in water, and <5 μg/L total urinary arsenic, n = 8). Immune profiling was performed using cryopreserved lymphocytes from the first 116 of the 129 remaining deliveries with available cord blood samples. Our study included pregnant women screened for the following eligibility criteria at their first prenatal visit: (1) currently pregnant, (2) 18 to 45 years old, (3) receiving routine prenatal care at one of the study clinics, (4) living in a household served by a private well (defined as a well serving fewer than 15 households or 25 individuals), (5) residing in the same place since their last menstrual period and using the same water supply, and (6) not planning to move prior to delivery. As only a small fraction of our study population is non-English speaking, we limited our recruitment to English speaking women.

2.3. Data collection

Women who consented were asked to complete a self-administered lifestyle and medical history questionnaire that covered socio-demographic factors such as age, race/ethnicity (of the mother and child's father), and level of education. Women were asked about their general health and medical history, reproductive history, as well as lifestyle and exposure questions, including tobacco and alcohol use. Trained study staff also performed a structured review of the prenatal and delivery medical records (maternal and infant) to document pregnancy and delivery information.

2.4. Assessment of arsenic exposure

We ascertained information about each participant's household water supply including the type of system (*e.g.*, drilled *versus* dug well, or spring), the use of water filters and the average number of cups per day of water participants consumed (in food or drink) from their household tap and other sources. Participants completed a diary of water, fish