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Differential sensitivities of bone marrow, spleen and thymus to genotoxicity induced by environmentally relevant concentrations of arsenite

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HIGHLIGHTS

- MMA^{+3} is the main species in bone marrow and thymus in As^{+3} exposed mice.
- Bone marrow is the most sensitive lymphoid tissue to arsenic-induced genotoxicity.
- Increase in DNA damage is correlated with more intracellular MMA^{+3} in bone marrow and thymus.
- An *in vivo* exposure to 100 ppb As^{+3} induced genotoxicity in bone marrow and thymus.
- MMA^{+3} is more genotoxic than As^{+3} *in vitro*.

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ABSTRACT

It is known in humans and mouse models, that drinking water exposures to arsenite (As^{+3}) leads to immunotoxicity. Previously, our group showed that certain types of immune cells are extremely sensitive to arsenic induced genotoxicity. In order to see if cells from different immune organs have differential sensitivities to As^{+3}, and if the sensitivities correlate with the intracellular concentrations of arsenic species, male C57BL/6J mice were dosed with 0, 100 and 500 ppb As^{+3} *via* drinking water for 30 d. Oxidation State Specific Hydride Generation- Cryotrapping- Inductively Coupled Plasma- Mass Spectrometry (HG- CT- ICP- MS) was applied to analyze the intracellular arsenic species and concentrations in bone marrow, spleen and thymus cells isolated from the exposed mice. A dose-dependent increase in intracellular monomethylarsonous acid (MMA^{+3}) was observed in both bone marrow and thymus cells, but not spleen cells. The total arsenic and MMA^{+3} levels were correlated with an increase in DNA damage in bone marrow and thymus cells. An *in vitro* treatment of 5, 50 and 500 nM As^{+3} and MMA^{+3} revealed that bone marrow cells are most sensitive to As^{+3} treatment, and MMA^{+3} is more genotoxic than As^{+3}. These results suggest that the differential sensitivities of the three immune organs to As^{+3} exposure are due to the different intracellular arsenic species and concentrations, and that MMA^{+3} may play a critical role in immunotoxicity.

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1. Introduction

Arsenic (As) contamination in food and drinking water is a world-wide public health issue. The trivalent inorganic form of As, arsenite (As^{+3}), is the most prevalent form in the environment. Exposure to As is associated with multiple diseases such as skin lesions, diabetes, cardiovascular diseases, and cancers (Argos et al., 2010; Schuhmacher-Wolz et al., 2009; Vahter, 2008). Once As^{+3} gets into the body, it is metabolized into monomethyl and dimethyl trivalent and pentavalent species (Aposhian and Aposhian, 2006). Monomethylarsonous acid (MMA^{+3}) has been shown to be more toxic than As^{+3} both *in vivo* and *in vitro* (Petrick et al., 2001; Styblo et al., 2000). MMA^{+3} can be further metabolized to

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dimethylarsonous acid (DMA^{+3}) or dimethylarsinic acid (DMA^{+5}) and excluded from the body.

Arsenic induced immunotoxicity has been studied by many groups both *in vitro* and *in vivo* (Ahmed et al., 2014; Nadeau et al., 2014; Biswas et al., 2008; Vahter, 2008; Soto-Peña et al., 2006; Gonsebatt et al., 1994). However, only a few studies have addressed the toxicity of As exposure to immune cells at environmentally relevant concentrations. Previous studies in our laboratory showed that *in vivo* drinking water exposure to As^{+3} at very low concentrations suppresses mouse bone marrow and spleen cell functions (Ezeh et al., 2014; Li et al., 2010). Human peripheral blood mononuclear cells (HPBMC) studies also showed a dose-dependent suppression of T cell proliferation at extremely low concentrations of As^{+3} (0.1–10 nM) in some individuals, with virtually all individuals susceptible to T cell immunosuppression by MMA^{+3} (Burchiel et al., 2014). Therefore, lymphocytes are extremely sensitive to As^{+3} exposure at environmentally relevant levels. However, it is unclear whether immunotoxicity is likely due to As^{+3} or MMA^{+3}.

T cells are generated in bone marrow and transferred to the thymus for development, while B cells develop in bone marrow. The spleen is a critical immune organ for the storage of both T and B cells and for systemic immune responses. In a previous study on the genotoxicity induced by As^{+3} in mouse thymus cells, we showed that mouse thymus cells are extremely sensitive to As^{+3} induced DNA damage, which is correlated with the inhibition of a base excision repair factor, Poly (ADP-ribose) polymerase (PARP) (Xu et al., 2016b). In the present study, the sensitivities of the three immune organs, bone marrow, spleen and thymus to As induced genotoxicity were compared both *in vivo* and *in vitro*. The species and levels of intracellular arsenic contents in the cells from the three immune organs were also determined and compared.

2. Methods

2.1. Chemicals and reagents

Sodium arsenite (CAS 774-46-5, $NaAsO_2$, Purity ≥90%) was purchased from Sigma-Aldrich (St. Louis, MO). Methylarsine iodide (MMA^{+3}) was obtained from Drs. Terry Monks and Todd Caminesch at the Southwest Environmental Health Sciences Center, University of Arizona. Penicillin/Streptomycin (Pen/Strep) and L-Glutamine were purchased from Life Technologies (Grand Island, NY). Dulbecco's phosphate buffered saline w/o Ca^{+2} or Mg^{+2} ($DPBS^{-}$) was purchased from Mediatech (Manassas, VA). Dimethyl sulphoxide (DMSO), RPMI 1640 and Iscove's Modified Dulbecco's Medium (IMDM) base medium were purchased from Sigma Aldrich. Fetal Bovine Serum (FBS) was purchased from Atlanta Biologicals (Flowery Branch, GA). Hanks Balanced Salt Solution (HBSS) was purchased from Lonza (Walkersville, MD). Sodium Hydroxide (NaOH) was purchased from EMD Chemicals Inc. (Gibbstown, NJ). 0.5M EDTA solution was purchased from Promega (Madison, WI). The Comet Assay kit (Cat. No. 4252-040-ESK), hOGG1 FLARE™ Assay kit (Cat. No. 4130-100-FK) and the PARP activity kit (Cat. No. 4685-096-K) were purchased from Trevigen (Gaithersburg, MD). The BCA assay kit (Cat. No. 23225) was purchased from Thermo Scientific (Rockford, IL).

2.2. *Mouse* in vivo *exposures*

C57BL/6J male mice were purchased at 8 weeks of age from Jackson Laboratory (Bar Harbor, ME). All animal experiments were performed following the protocols approved by the Institutional Animal Use and Care Committee at the University of New Mexico Health Sciences Center. Following one week of acclimation, mice (2–3 per cage) were exposed to As^{+3} at different concentrations *via* drinking water for 30 d. As^{+3} doses were prepared fresh weekly by weighing each water bag and determining the appropriate amount of stock As^{+3} solution to add to each bag to yield 100 and 500 ppb. No treatment was added to control bags. Water bags were weighed after each week and the change in weight was used to estimate the amount of water consumed by each group. The As^{+3} concentrations in drinking water were verified using Mass Spectrometry by Dr. Abdul-Mehdi S. Ali at Department of Earth and Planetary Sciences, University of New Mexico. Mice were fed 2020X Teklad global soy protein-free extruded rodent diet (Envigo, Indianapolis, IN) throughout the experiment.

2.3. Isolation of bone marrow cells

Bone marrow cells were isolated according to the procedures described in Ezeh et al. (2014). Basically, mouse femurs were collected into HBSS medium in our animal facility and transferred to our laboratory to extract cells. Femurs were placed in petri dish containing 5 ml of cold sterile bone marrow medium (500 ml IMDM with 2% FBS, 2 mM L-glutamine, and 100 mg/ml Pen/Strep) and trimmed to expose interior marrow shaft of the femur, the end of the femur were then cut off. one cc syringe and 25 gauge needle were used to flush the bone marrow medium through the femur several times to release cells into the petri dish. The suspension was immediately transferred to a 15 ml centrifuge tube, centrifuged at 200 × *g* for 10 min, aspirated, and washed with bone marrow medium. The cell count and viability were determined by acridine orange/propidium iodide (AO/PI) staining on a Nexcelom Cellometer 2000.

2.4. Isolation of thymus and spleen cells

Thymus and spleen were isolated following the sterile procedures described in Xu et al. (2016b). Basically, mouse thymus and spleen were harvested in our animal facility and transferred to the laboratory in HBSS on ice. Single cell suspensions of spleen and thymus cells were prepared by homogenizing the organ between the frosted ends of two sterilzed microscope slides (Fisher Scientific, Pittsburgh, PA) into a dish containing 5 ml of cold mouse medium (500 ml RPMI 1640 with 10% FBS, 2 mM L-glutamine, and 100 mg/ml Pen/Strep). Suspended cells were centrifuged at 200 × *g* for 10 min, aspirated, and washed with mouse medium. The cell count and viability were determined by AO/PI staining on a Nexcelom Cellometer 2000.

2.5. Oxidation state specific hydride generation- cryotrapping-inductively coupled plasma- mass spectrometry (HG- CT- ICP- MS)

The analysis of tri- and pentavalent As species was performed by HG-CT-ICP-MS as previously described (Currier et al., 2014; Matoušek et al., 2013). Briefly, cell pellets were lysed in ice-cold deionized water. The trivalent species, As^{3+}, MMA^{3+}, and dimethylarsinite (DMA^{3+}) were measured in an aliquot of cell lysate directly, without pretreatment. Another aliquot was treated with 2% cysteine and analyzed for total inorganic As ($As^{3+} + As^{5+}$), total methyl-As ($MMA^{3+} + MMA^{5+}$), and total DMAs ($DMA^{3+} + DMA^{5+}$). Calibration curves were generated using cysteine-treated pentavalent As standards, (at least 98% pure) as previously described (Hernández-Zavala et al., 2008). The concentrations of pentavalent As species were determined as a difference between the values obtained for cysteine-treated aliquots and values from untreated sample aliquots. The instrumental LODs for As species analyzed by HG-CT-ICP-MS ranged from 0.04 pg As for methylated arsenicals to 2.0 pg As for inorganic arsenicals. All values are expressed as pg of As in each arsenic species.

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