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## **Arsenic and the immune system**

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## **Abstract**

Arsenic is a metalloid to which millions of individuals are exposed worldwide, primarily through the consumption of contaminated drinking water or food items. Chronic arsenic exposure is associated with an increased incidence of several diseases, including lung infections and skin cancers. Epidemiological and experimental evidence demonstrates that the metalloid impairs the activity of both the innate and adaptive immune systems. Arsenic alters the differentiation, activation and/or proliferation of human macrophages, dendritic cells and T lymphocytes. Arsenic immunotoxicity results from intracellular oxidative lesions modulating basal gene expression or leading to DNA damage. Arsenic also induces epigenetic effects by modulating DNA methylation and post-translational histone modifications. In addition, the metalloid has recently been found to inhibit *in vitro* and *in vivo* inflammasome activities. Arsenic immunotoxicity likely contributes to the systemic effects associated with arsenic exposure by limiting immune surveillance and/or promoting inflammation. Experimental studies also suggest that the immunosuppressive effects of arsenic efficiently prevent or treat severe immune-related diseases. The purpose of this review is to summarize the current knowledge of the impact of arsenic on the immune system.

Keywords: arsenic, immunity, cell stress, DNA damages, epigenetic, immunosuppression

## 1. Introduction

Arsenic is a toxic metalloid naturally present at high levels in the earth's crust in many countries worldwide. Arsenic is complexed to a sulphur atom in various mineral ores, such as arsenopyrite, orpiment and realgar. Physical, chemical and/or microbial changes in the soil can promote arsenic mobilization from minerals and its accumulation in groundwater. The consumption of contaminated drinking water is the first source of arsenic exposure for millions of individuals in several Asian and American countries [1]. Since contaminated groundwater can be used to irrigate and cook cereal crops and other food items, humans can also be exposed to arsenic through the ingestion of vegetables, fruits, grains and rice [2]. In addition, arsenic is used for various industrial activities, including the production of wood preservatives, electronics and pesticides, which can induce occupational exposures [3].

Arsenic speciation includes several inorganic and organic species in different oxidation states. Contaminated drinking water, for example, contains inorganic pentavalent and trivalent forms of arsenic, which are largely absorbed from the human gastrointestinal tract, likely through glucose, phosphate or organic anion transporters and aquaporins. In contrast to mercury or cadmium, arsenic is a metalloid that does not chronically accumulate in specific organs. This compound is rapidly metabolized in the liver through biological methylation, oxidation/reduction and glutathione conjugation. Arsenic biotransformation generates reactive mono- and -dimethylated metabolites that likely contribute to its systemic effects [2].

Arsenic toxicity has been extensively reviewed in several recent articles [1–3]. Chronic ingestion of low concentrations of arsenic initially induces skin lesions but can be toxic to many other organs [4]. Long term effects primarily include cutaneous, respiratory, neurodevelopmental, hepatic, endocrine and cardiovascular disorders [4]. This metalloid is classified by the IARC as a carcinogenic compound for humans (group 1), and epidemiological studies have clearly demonstrated that arsenic significantly increases the incidence of skin, bladder and lung cancers [3]. In addition, arsenic modulates the activity of both innate and adaptive immunity by various cellular and molecular mechanisms.

Immunomodulation likely promotes arsenic systemic toxicity, which results from environmental or occupational exposure. However, specific immunosuppressive effects of arsenic may also be beneficial for the treatment of certain immune-related diseases. Thus, the purpose of this review is to summarize our current knowledge on arsenic immunotoxicity.

## **2. Effects of arsenic on immune cells**

### **2.1 Macrophages and dendritic cells (DCs)**

Macrophages and DCs are professional phagocytes that control innate immunity and regulate immune surveillance against infections and cancers. Monocytes constitute major precursors of both macrophages and DCs, especially during inflammatory states. Monocytes migrate in tissues where they differentiate into mature phagocytes. A primary function of macrophages is to engulf and digest microbes, cellular debris and cancer cells by phagocytosis. In response to infection or tissue damage, macrophages secrete a large spectrum of cytokines according to their local environment. Chronic exposure to arsenic significantly impairs the *ex-vivo* differentiation of peripheral blood monocytes into mature macrophages. Banerjee et al. [5] reported that monocyte-derived macrophages from arsenic-exposed individuals with skin lesions exhibited reduced cell adhesion capacity, F-actin expression, nitric oxide production and phagocytic activity. *In vitro*, 0.5 to 2  $\mu\text{M}$  arsenic trioxide (ATO) was shown to block the differentiation of human peripheral blood monocytes into functional macrophages by repressing NF- $\kappa$ B-related survival pathways [6]. Short treatments of human mature macrophages with non-cytotoxic arsenic concentrations (1 to 5  $\mu\text{M}$ , 6 h) was shown to inhibit the transcriptional activity of the liver X receptor, resulting in the decreased expression of its target genes, ABCA1 and SREBP-1c, and impaired cholesterol efflux [7]. Prolonged treatment (3 to 6 days) with ATO was shown to de-differentiate macrophages into CD14-expressing monocytic-like cells, notably through the reorganization of the actin cytoskeleton [8]. Metalloid-exposed macrophages display increased secretion of the pro-inflammatory chemokines CXCL2 and CCL18 and a decreased expression of several macrophage markers, including metalloproteinase 7, 9 and

12 [8,9]. Moreover, these macrophages exhibit an enhanced pro-inflammatory response to the lipopolysaccharide (LPS) endotoxin. Increased exposure to arsenic through contaminated drinking water is also positively correlated with high CD14 expression on monocytes and with elevated plasma levels of pro-inflammatory cytokines, including TNF $\alpha$ , and IL-8 [10,11].

Recent studies have shown that arsenic alters the functions of microglia, which are the resident macrophages found in the central nervous system [12,13]. The metalloid notably increased *ex-vivo* secretion of IL-6 and TNF- $\alpha$  from microglia isolated from mice exposed for 7 days to 0.38 mg/kg arsenite [12]. *In vitro*, the supernatant of arsenic-exposed-microglial cultures induced murine neuronal cell death [14]. The death process did not result from inflammation but was, rather, caused by a critical cystine/glutamate imbalance induced by arsenic in the microglia. These specific effects may contribute to the increased incidence of developmental neurotoxicity in children chronically exposed to arsenic [15].

DCs are primary antigen-presenting cells that link innate immunity to adaptive immunity. Once activated by infectious agents or tumor cell antigens, DCs migrate in the peripheral lymph nodes where they activate T cells and promote their polarization. At low concentrations (0.1 to 0.5  $\mu$ m), arsenite does not block the ability of mature DCs to activate the proliferation of human T cells *in vitro*, but it significantly inhibits the secretion of IL-12p70 and IL-23, two interleukins that enhance T cell polarization [16]. At concentrations higher than 1  $\mu$ M, arsenic blocks myeloid DC differentiation by inducing monocyte necrosis [16], and it decreases the phagocytic activity of immature DCs [17]. These *in vitro* experimental results need to be confirmed by investigating DC functions in chronically exposed individuals. Nevertheless, various reports have shown that quantitative losses and structural lesions of Langerhans cells, which are rather similar to dermal DCs, are specifically detected in the skin lesions of exposed individuals [18,19]. Although arsenic can clearly alter DC-induced T cell activation, a specific effect of the metalloid on antigen presentation by DCs remains to be demonstrated.

## 2.2 Lymphocytes

T and B lymphocytes mediate the cellular and humoral responses of adaptive immunity, respectively. The activation of T lymphocytes by DCs stimulates their proliferation and differentiation into various CD4<sup>+</sup> T helper cell subtypes and CD8<sup>+</sup> T cytotoxic cells. Numerous studies have demonstrated that arsenic markedly alters both the number and function of human T lymphocytes. Specifically, chronic arsenic exposure is correlated with decreased percentages of peripheral blood CD4<sup>+</sup> T cells and CD4/CD8 ratios in children and adults and with reduced *ex-vivo* proliferation of activated T lymphocytes [20–22]. Wu et al. [10] reported that the expression of various pro-inflammatory cytokines, growth factors and chemokines were upregulated in non-stimulated blood lymphocytes isolated from high arsenic-exposed individuals. The transcript levels of IL-1 $\beta$ , IL-6, chemokine C-C motif 2/monocyte chemoattractant protein-1 (CCL2/MCP-1), chemokine C-X-C motif ligand 1/growth-related oncogene alpha (CXCL1/GRO1), CXCL2/GRO2 and matrix metalloproteinase 1 (interstitial collagenase) were significantly increased in lymphocytes from individuals with the highest arsenic blood concentrations (9.6 to 46.5  $\mu\text{g/L}$ ). Nevertheless, the *ex-vivo* secretion of IL-2 from lymphocytes activated with phytohemagglutinin or concanavalin-A was negatively correlated with urinary arsenic levels [20,21]. *In vitro* experiments have confirmed that low and non-cytotoxic arsenite concentrations significantly repress both the proliferation and IL-2 secretion of human activated T cells [23,24]. Moreover, studies have revealed that the metalloid selectively inhibits the differentiation of T helper 17 lymphocytes by specifically repressing IL-17 expression, a cytokine that increases anti-infectious defenses but also promotes inflammation in several auto-immune diseases [25]. The *In vitro* inhibition of T cell proliferation is sex-dependent, i.e., the inhibition is more potent in peripheral blood lymphocytes isolated from females [26]. *In utero*, arsenic exposure also decreases the percentage of CD4<sup>+</sup> T cells in the cord blood [27] and alters the thymic function in newborns [28]. In fact, recent studies carried out on murine models showed that chronic arsenic exposure affects the differentiation of T cell precursors in the thymus. Exposure to low doses of arsenite (< 0.1 mL/L) in drinking water for 30 days increases the numbers of CD4<sup>+</sup> CD25<sup>+</sup>

FoxP3-expressing cells, also known as natural regulatory T (nTreg) cells, in the thymus of male Swiss albinos mice [29]. Oral arsenite gavage (0.038 to 3.8 ppm) of male Balb/c mice for 30 days specifically promotes the CD4 lineage in the thymus without affecting the tissue ultrastructure [30]. However, this treatment also triggers the differentiation of splenic CD4+ T cells into nTreg cells and induces immunosuppression. Further research is thus required to determine the role of nTreg cells in the immunotoxic effects of arsenic in humans.

B lymphocytes, or B cells, mediate humoral immunity by producing and secreting antibodies. In contrast to T cells, the impact of arsenic on human B cells remains to be fully characterized. At low concentrations (0.1 to 2  $\mu\text{M}$ ), arsenite has either no effect or modest effects on the *in vitro* CD40L-dependent Immunoglobulin M antibody-forming cell response of human isolated naïve B lymphocytes, whereas this concentration of arsenite markedly reduces the same response of murine B cells [31]. Arsenite and its monomethylated trivalent metabolite, monomethylarsonous acid, have also been demonstrated to reduce *in vivo* and *in vitro* murine pre-B cell development from hematopoietic stem cells, likely by interfering with IL-7 signaling, and to repress murine humoral immunity [32,33]. It will be interesting to confirm these results with human B cells.

### **3. Molecular basis of arsenic immunotoxicity**

#### **3.1 Oxidative stress and DNA damage**

Arsenic is a pro-oxidant metalloid that forms stable complexes with vicinal thiol residues in several proteins and increases the levels of reactive oxygen (ROS) in several cell types, including macrophages and lymphocytes [8,34]. Previous reports suggest that the effects of arsenic on the viability and functions of macrophages, DCs or lymphocytes can result from oxidative stress and related DNA damage. In this context, at non cytotoxic concentrations (< 1  $\mu\text{M}$ ), ATO produces ROS in human monocyte-derived macrophages by directly activating NADPH oxidase at the plasma membrane [8]. To stimulate this superoxide-generating enzymatic complex, the metalloid induces the phosphorylation and subsequent membrane translocation of the p47<sup>phox</sup> NADPH oxidase partner through a Rho-kinase/p38-kinase-



dependent signaling pathway. Arsenic may also produce ROS and disrupt macrophage functions by inducing the unfolded protein response [35]. At a higher concentration (5  $\mu$ M), ATO significantly reduces the mitochondrial transmembrane potential, increases ROS levels and triggers caspase-dependent apoptosis in human CD4<sup>+</sup> and CD8<sup>+</sup> T cells [36]. ROS likely contribute to the DNA damage detected in peripheral blood lymphocytes either isolated from arsenic-exposed individuals or treated *in vitro* with arsenical compounds, by eliciting 8-hydroxy-2'-deoxyguanosine adducts and/or repressing DNA repair [34,37,38]. In addition to inducing DNA damage, arsenic impairs gene expression in human immune cells by modulating redox-sensitive signaling pathways. Notably, prevention of oxidative stress by an NADPH oxidase inhibitor or an antioxidant blocks CCL18 and CXCL2 gene expression in arsenic-exposed macrophages [8,39]. In addition, in response to cell stress, the transcription factor NRF2 not only regulates the induction of several antioxidant genes but also mediates the arsenic-dependent inhibition of IL12 gene expression in human mature DCs [40].

### **3.2. Epigenetic effects**

Molecular epidemiological studies have suggested that arsenic can regulate gene expression in white blood cells by modifying the epigenome. Increased arsenic exposure through consumption of contaminated drinking water has been positively associated with global DNA methylation in the peripheral blood mononuclear cells of Bangladeshi adults [41,42]. Niedzwiecki et al. (2013) notably reported that the highest DNA methylation levels are measured in individuals with the highest arsenic exposure [42]. However, subtle modifications of DNA methylation can also be detected at the gene level. Indeed, in chronically exposed individuals, increased arsenic exposure either reduces or enhances the methylation levels of various genes involved in major cell functions, such as lipid metabolism, intracellular pH regulation or the nuclear factor  $\kappa$ B signaling pathway [43]. Similarly, Engström et al. [44] reported that increased arsenic exposure was associated with significant alterations of the CD4<sup>+</sup> T cell methylome in Argentinean women. Both hypo- and hyper-methylated regions were detected in genes regulating T cell activation and differentiation.

Interestingly, among these genes, Engström et al. found that the interferon regulatory factor 1 (IRF1) gene was hyper-methylated and its expression was decreased. In contrast, the ras homolog family member H (RHOH) gene was hypo-methylated and its expression was upregulated. Although these results suggest that arsenic exposure may alter gene expressions by differentially modulating gene methylation levels, additional studies are warranted to assess the causality and clinical significance of this phenomenon. In addition to DNA methylation, other post-translational histone modifications have also been identified in peripheral blood mononuclear cells of adults exposed to arsenic [45,46]. Notably, Chervona et al. (2012) reported that in Bangladeshi adults exposed to water containing up to containing 500 µg/L arsenic, total urinary arsenic concentration was positively correlated with the level of histone 3 lysine 9 di-methylation (H3K9me2) and inversely correlated with H3K9 acetylation [45]. Other post-translational histone modifications associated with arsenic exposure differed in a sex-dependent manner. For example, arsenic concentration in water was positively associated with H3K4me3 and H3K27me3 among females but negatively correlated with the same modifications among males. Finally, Pournara et al. (2016) reported that the impacts of arsenic on histone residues could also differ between CD4+ and CD8+ T cells [46]. Increased arsenic exposure in drinking water was negatively correlated with H3K9me3 in CD4+ T cells but not in CD8+ T cells. Metalloid effects on histone residues are thus complex, depending on the cell types and sex, and these effects need to be further explored for a better understanding of their biological significance.

### **3.3. Inhibition of inflammasomes**

Inflammasomes are cytoplasmic multiprotein complexes that specifically control the activation of the caspase 1-dependent cleavage of the pro-inflammatory cytokines IL-1 $\beta$  and IL-18 into their active forms. These complexes are primarily generated in macrophages exposed to pathogen-derived stimuli (bacterial toxins), self-derived molecules (uric acid, ATP) and environmental contaminants (silica, asbestos) [47]. They are composed of nucleotide-binding oligomerization domain-like receptor (NLR) proteins that act as stimuli

sensors. *In vitro*, arsenite and ATO inhibit the NLRP1, NLRP3 and NAIP5/NLRC4 inflammasomes in murine bone marrow-derived macrophages exposed to their respective activating stimuli [48]. Maier et al. [48] demonstrated that, *in vivo*, arsenite delays the NLRP1-mediated death of mice exposed to anthrax lethal toxin and reduces NLRP3-dependent inflammation in mice treated with monosodium urate crystals. Arsenic blocks the maturation of pro-IL-1 $\beta$  by preventing the autoproteolytic and substrate cleavage activities of caspase-1 in murine macrophages [48]. Low concentrations of ATO (0.1 to 1  $\mu$ M), which fail to repress the upregulation of IL-1 $\beta$  mRNA caused by LPS in human monocyte-derived macrophages (Figure 1A), inhibit the silica-induced secretion of active IL-1 $\beta$  in the macrophage supernatant (Figure 1B). These results suggest that ATO directly impairs the inflammasome activity of human macrophages, i.e., it suppresses the cleavage of pro-IL-1 $\beta$ . Inflammasome complexes may thus constitute important molecular targets of arsenic in humans.

#### **4. Consequences of arsenic immunotoxicity in humans**

##### ***4.1 Contribution to diseases associated with chronic environmental exposure***

As described above, arsenic exerts pleiotropic effects on immune cells, which may initially result in the suppression of major immune functions. Notably, arsenic decreases bacterial phagocytosis by macrophages, reduces T cell proliferation and markedly represses the secretion of pro-inflammatory cytokines by activated DC and T cells. These inhibitions can increase the susceptibility to infection by limiting pathogen eradication. Arsenic-induced immunosuppression likely increases the incidence of lower respiratory tract infections and diarrhea, which frequently develop in young exposed children from low-income countries [49,50]. Particularly, increased arsenic exposure was found to promote pulmonary tuberculosis in Chile [50] and visceral leishmaniasis in India [51]. In a murine model, chronic exposure to low doses of arsenic also increased morbidity of influenza A infection by repressing DC-mediated immune responses in mice [52]. In addition, immunosuppression impairs the surveillance and killing of tumorigenic cells, which may promote lung and skin

cancer associated with arsenic exposure [53]. Conversely, arsenic may induce a basal inflammatory state by increasing the secretion of molecules such as TNF- $\alpha$  and IL-8 from monocytes/macrophages and lymphocytes. Chronic inflammation and secretion of growth factors, such as IL-8, can also establish microenvironments supportive of tumor development [54]. Epidemiological studies carried out in various countries demonstrate that arsenic significantly increases the risk of atherosclerosis [55,56]. Several mechanisms may contribute to the development of atherosclerosis, including general pro-inflammatory cytokine secretion [10], increased ROS production [8], enhanced monocyte adhesion to vascular endothelium [57] and reduced cholesterol efflux from macrophages [7]. In murine models, at least, methylated metabolites and arsenic-3-methyltransferase play major roles in these pro-atherogenic effects of arsenic [58].

#### *4.2. Potential beneficial effects of arsenic-induced immunosuppression*

Recently, various experimental studies have suggested that the immunosuppressive properties of arsenic may reduce or treat severe immune-related diseases. Indeed, ATO prevents the development of a lupus-like syndrome in MRL/lpr mice by eliminating activated T cells that cause lymphoproliferation and skin, lung and kidney damage [59]. ATO also abrogates chronic sclerodermatous graft-versus-host disease that follows allogenic hematopoietic stem cell transplantation in a murine model [60]. The metalloloid inhibits the alloreactive process by selectively killing activated DCs and CD4-positive T cells. In addition, ATO prolongs cardiac and islet allograft survival in acute rejection murine models through the repression of alloreactive CD4+ and CD8+ memory T cells [61–63]. Finally, the fact that arsenic blocks inflammasome activity and the subsequent IL-1 $\beta$  production in human macrophages suggests that arsenic may limit chronic inflammation in severe inflammasome-mediated disease [47].

## **5. Conclusions**

Many human population-based and/or experimental studies strongly suggest that arsenic exerts potent immunotoxic effects towards key human immune cells, such as macrophages,

DCs and T lymphocytes (Figure 2). Multiple molecular and cellular mechanisms can mediate arsenic immunotoxicity, including ROS production, alteration of redox-sensitive signaling pathways, DNA damage, epigenetic effects and inflammasome inhibition. Arsenic immunotoxicity likely contributes to the systemic deleterious effects associated with chronic environmental exposure. Notably, arsenic-induced immunosuppression may favor the development of infectious diseases and cancers. On the other hand, data derived from experimental studies using various mouse models support the hypothesis that the immunosuppressive effects of arsenic may be beneficial for limiting the exacerbation of the immune system in severe immune-related diseases. However, further research is still required to determine the role of arsenic immunotoxicity in the systemic effects of arsenic exposure and to investigate the potential use of this metalloid in medical immunosuppressive therapy.

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- of outstanding interest

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The investigators demonstrate that trivalent inorganic arsenic ( $\text{As}_2\text{O}_3$ , 0.25-2  $\mu\text{M}$ ) totally inhibits expression and secretion of IL-17A from naive and memory human CD4+ T lymphocytes differentiated into Th17 cells, notably by impairing JNK/C-jun-dependent signalling and RORC- $\gamma$ t expression. IL-17A, which plays a key role in anti-infectious defenses but also in the development of various chronic inflammatory diseases, may thus constitute a major target of the arsenic in humans.

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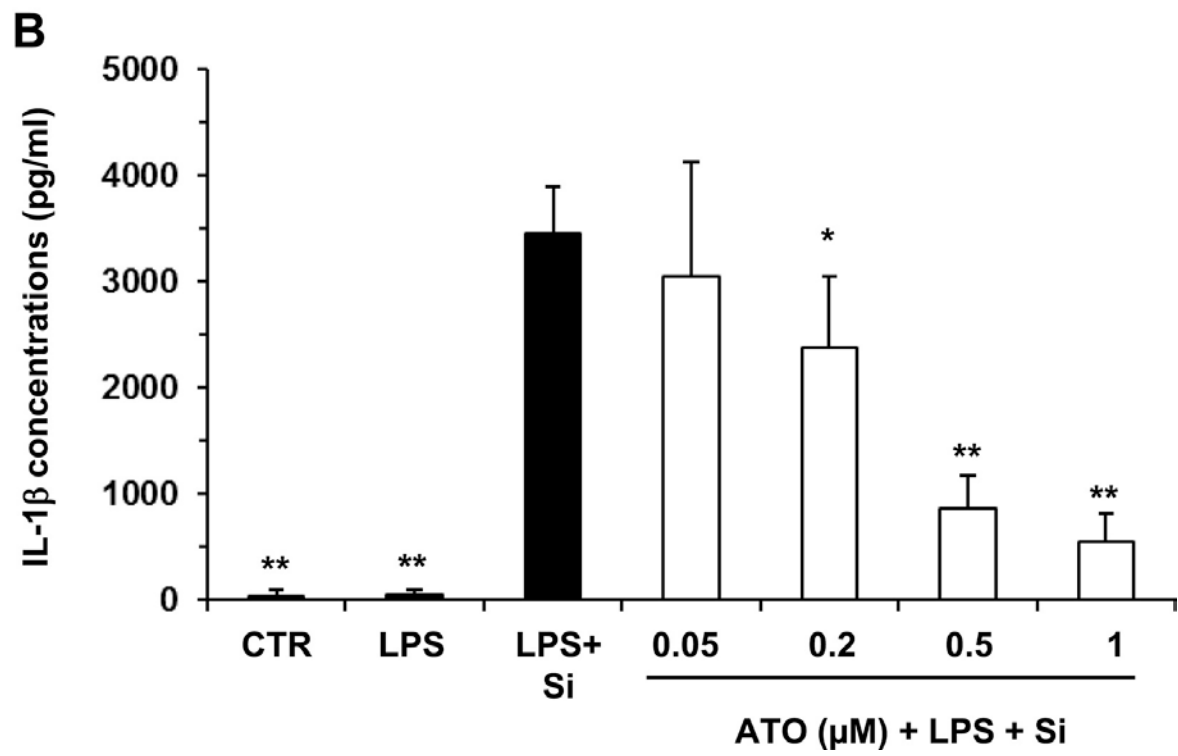
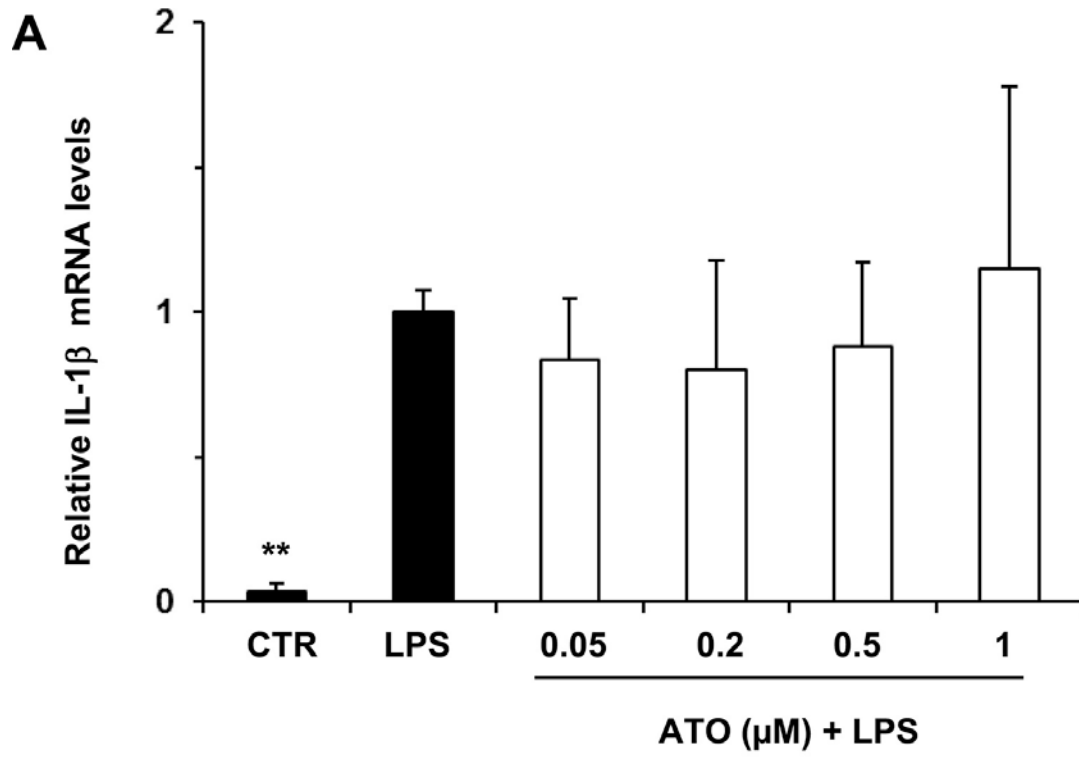
## Legends

**Figure 1.** Arsenic trioxide (ATO) inhibits IL-1 $\beta$  secretion from human macrophages without impairing IL-1 $\beta$  mRNA levels. Following the isolation of peripheral blood mononuclear cells by Ficoll gradient centrifugation, human monocytes were selected by an adhesion step and then differentiated into macrophages in RPMI medium containing 50 ng/mL macrophage-colony stimulating factor for 6 days. Macrophages were left untreated (CTR) or pre-treated with ATO for 2 h before stimulation with 20 ng/mL LPS (*Escherichia coli* 055:B5, Sigma Aldrich, France) for 4 h to increase IL-1 $\beta$  mRNA expression. Next, cells were harvested (A) or cultured with 0 or 100  $\mu$ g/mL silica (Si) (DQ12 mineral dust silica, DMT GMBH, Germany) for 16 h (B) to activate NLRP3 inflammasome and produce active IL-1 $\beta$  protein. At the end of the treatment, the culture media were collected and the levels of active IL-1 $\beta$  secreted in the supernatant were quantified by ELISA (Duoset ELISA development system kits, R and D system, France) (B). In (A), mRNA levels were determined by RT-qPCR using the fluorescent dye SYBR Green methodology and a CFX384 real-time PCR system (Bio-Rad). Data are presented relative to mRNA levels found in macrophages treated with LPS and arbitrarily set at the value of 1. Results in (A) and (B) are expressed as the means  $\pm$  SD of 6 independent experiments. Significant differences were evaluated using ANOVA followed by the multi-range Dunett's t test for multiple comparisons. The criterion for significance of the difference between means was \* $p < 0.05$  or \*\* $p < 0.01$ , versus LPS (A) or LPS+Si (B).

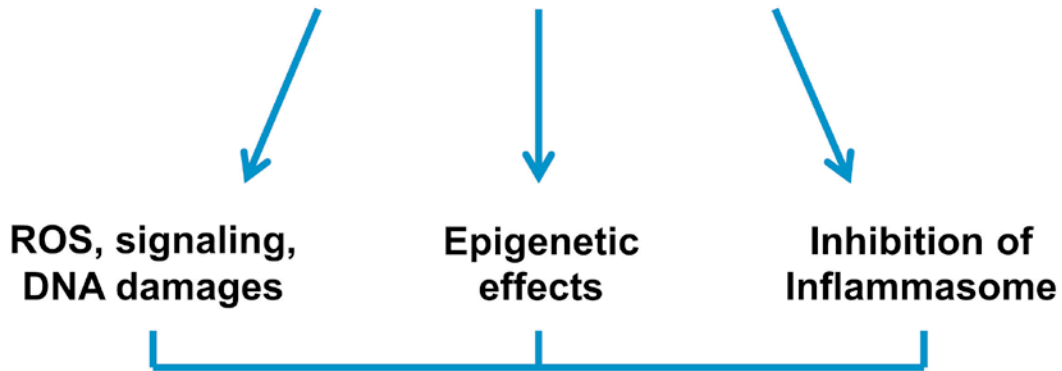
**Figure 2.** Effects of arsenic on immune cells and their potential consequences for humans. Environmental or occupational exposure to arsenic can impact the differentiation and activation of macrophages, dendritic cells and T lymphocytes. Arsenic impairs immune cell functions by several molecular mechanisms, including the production of reactive oxygen (ROS), modulation of redox-sensitive signaling pathways controlling gene expression, induction of DNA damage, epigenetic effects (DNA methylation and histone modifications), and the inhibition of the inflammasome. Arsenic immunotoxicity may contribute to the increased incidence of respiratory infections, diarrhea, lung and skin cancers, and



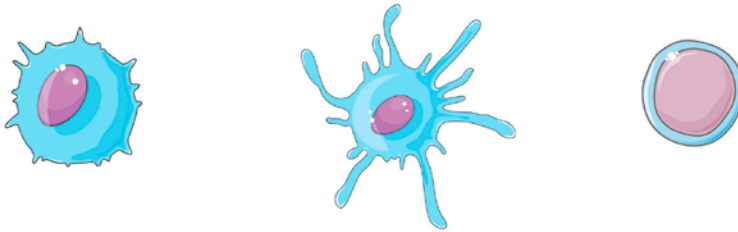
atherosclerosis in environmentally exposed individuals. In contrast, experimental studies have demonstrated that the immunosuppressive effects of arsenic may counteract hyperactivity of the immune system in murine models of lupus, chronic sclerodermatous graft-versus-host disease and acute allograft rejection. Moreover, these effects may prevent NLRP3-dependent inflammation from developing in mice exposed to monosodium urate crystals. These results support the idea that arsenic may have beneficial therapeutic immunosuppressive effects for the treatment of certain human diseases.



## **Exposure to arsenic**



**Alteration of the differentiation and activation of  
macrophages, dendritic cells and/or T lymphocytes**



**Potential consequences of**

