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## REVIEW ARTICLE

# Aberrant immune responses in arsenical skin cancers

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Received 4 May 2009; accepted 18 November 2010

Available online 6 July 2011

**KEYWORDS**Arsenic;  
Bowen's disease;  
Carcinogenesis;  
Immune system

**Abstract** Arsenic is a well-known human carcinogen. It also impairs immune functions and activation in many aspects. However, only a small portion of arsenic-exposed population develops skin abnormalities, including Bowen's disease and skin cancers. Differential immune activation among the individuals might account for the different susceptibilities. In patients with arsenic-induced Bowen's disease, there is a selective CD4 T-cell apoptosis through tumor necrosis factor-alpha pathway, decrease in macrophage differentiation and phagocytosis, reduced Langerhans cell numbers and dendrites, altered regulatory T-cell distribution, and other immune alterations. Several lines of evidence from mouse and fish studies also confirmed the potent and multifaceted effects of arsenic in the immune system. The molecular bases of immunosuppression by arsenic in lymphocytes may include chromosomal and DNA abnormalities, decreased T-cell receptor activation, and the cellular status of oxidation and methylation. This article also reviews the causative and differential role of selective CD4 cell apoptosis and the carcinogenesis of arsenic-induced Bowen's disease.

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**Introduction**

Arsenic accumulation in the environment has been intensified by human activities, such as fossil fuel combustion, metal smelting and mining, and semiconductor and glass industries [1]. Interestingly, only around 10% of the population exposed to arsenic develop skin abnormalities,

including hyper-/hypopigmentations, arsenic keratosis, Bowen's disease, and invasive skin cancers. Among these people exposed to arsenic, only up to 1% of the population develops skin cancers. Personal genetic variability, immune system, and the interaction of both might differ, leading to differential susceptibilities. Long-term arsenic exposure results in impaired immunity in some susceptible individuals. The decrease in contact hypersensitivity reaction is the hallmark of decrease in cellular immunity and occurs in patients with arsenic-induced Bowen's disease. Bowen's disease, a skin carcinoma *in situ*, can occur in any area of the skin. Clinically, arsenic-induced Bowen's disease tends

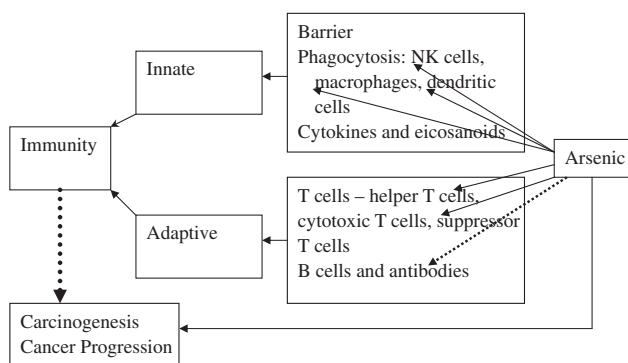
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to be multifocal and distributed in sun-protected skin, whereas classical Bowen's disease tends to be solitary and mainly located in sun-exposed skin. Histopathologically, there is no difference between arsenic-induced and classical Bowen's disease. There are full layers of epidermal dysplasia, hyperkeratosis, acanthosis, individual dyskeratosis, and cellular dysplasia. Arsenic, as an immunotoxin, is also the therapeutic choice for acute promyelocytic leukemia, a special form of hematological malignancy from myelocyte series. This article reviews the impact of arsenic on immune system as a whole and the interaction of selective immune suppression and the occurrence of skin cancers in arsenic-exposed patients.

## Overview of immunity and tumor immunity

Immune system is vital to fight against foreign pathogens, to clear endogenous tumor antigens, and to recognize self and foreign identities. It functions like a military force in the body to fight against different enemies in different ways. Immunity confers sufficient biological defenses to avoid infection, disease, or cancers. Immune system could be classified as specific and nonspecific components (Fig. 1). The nonspecific, or innate, components act either as barriers or as passive eliminators of a wide range of pathogens, irrespective of antigenic specificity. Other specific or adaptive components are able to generate pathogen-specific immunity each time they encounter new pathogens. The innate immune response is activated as the first line of defense of the host and is a prerequisite of potentiating the adaptive immune responses. Another classification of immune system depends on the participation of cellular immune component. Either innate or adaptive immune system could be classified into cellular or humoral immunity that indicates the involvement of immune cells or the antibodies, respectively.

The host immune system clears tumor antigens either through natural killer cells or the cytotoxic T cells ( $CD8^+$ ) with the help of helper T ( $CD4^+$ ) cells. On the other hand, tumors may evade host immune surveillance by secreting anti-inflammatory mediators, such as transforming growth factor-beta, or direct the development of regulatory T cells. Without an intact or a functional immune system, apparently, the opportunistic infection will occur. An example of chronic immunosuppression is seen in patients



**Figure 1.** The functional components of immune system. NK = natural killer.

with renal transplantation on immunosuppressive drugs. These patients tend to have increased risk of developing multiple skin cancers [2].

## Arsenic induces aberrant innate and adaptive immune responses

Arsenic exposure in mice diminished the formation of immunoglobulin M and immunoglobulin G, antigen-mediated T-cell proliferation [3,4], and macrophage phagocytotic activity and their nitric oxide release capacity as well [5]. It also decreased  $CD4^+$  cell numbers from spleen and suppressed contact hypersensitivity responses [6]. Of note, arsenic-treated mice demonstrated a significantly decreased resistance to the B16F10 melanoma with a sevenfold increase in tumor burden at a dose of 200 mg/kg. Studies of rodents in assessing human arsenic toxicity are limited in human implications because arsenics are proved to be human Class IA carcinogen but not a carcinogen in rodents. In fact, a high concentration of arsenic is usually required to have significant biological effects on rodents. Nonetheless, a breakthrough of the mouse study in arsenic is by Waalkes' group that has demonstrated the transplacental carcinogenesis in mice [7,8]. Male C3H mice exposed to arsenic *in utero* developed liver carcinoma and adrenal cortical adenoma during adulthood. Prenatally exposed female C3H offspring showed increases in ovarian tumors, lung carcinoma, and proliferative lesions of the uterus and oviduct. In addition, prenatal plus postnatal arsenic exposure to the tumor promoter, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), in C3H mice produces excess lung tumors in both sexes and liver tumors in females [7,8]. However, fetal arsenic exposure does not increase the risk of skin cancers unless the strain had genomic susceptibility (TgAC mice) and when TPA was also applied, implicating stem cells as a target of arsenic in the fetal basis of skin cancer in adulthood [9]. Although the immune status of these offspring mice was not checked, the epidemiological studies have declared that *in utero* arsenic exposure impaired child thymic development and enhanced morbidity, probably through immunosuppression [10].

Not only are the immune responses of rodents altered by arsenic but also the innate immune system was suppressed in zebrafish [11]. Exposure to 2-ppb and 10-ppb arsenic, both considered safe levels in drinking water, resulted in a greater than 50-fold increase in viral load and at least a 17-fold increase in bacterial load in embryos of zebrafish. Declines of proinflammatory cytokines were noticed, including tumor necrosis factor-alpha (TNF-alpha), IL-1 beta, and interferon. The utilization of zebrafish in arsenic toxicity has the advantages of short study time, *in vivo* demonstration, and impact on embryonic development. In a study from catfish, leukocyte counts from spleen and total head kidney were reduced in catfish exposed to 42  $\mu$ M arsenic, 1/50<sup>th</sup> of the toxic dose [12]. Of note, bacterial phagocytosis was interfered with and the immune status to *Clarias batrachus* was weakened. Thus, organ- and cell-specific effects by arsenic are demonstrated in the immune functions of catfish.

Monocytes and macrophages may also constitute the major targets of arsenic. Differentiation between

monocytes and macrophages in humans is impaired by arsenic. Arsenic induced a rapid cell rounding, loss of adhesion, and actin reorganization, mainly through Ras homolog gene family member A-associated kinase pathway [13]. Thus, human macrophages are also sensitive targets of arsenic immunotoxicity.

Low concentrations (<5  $\mu\text{M}$ ) of arsenic tended to increase the number of natural T-regulatory (nTreg) lymphocytes from normal controls. However, in healthy subjects chronically exposed to arsenic, urinary arsenic levels are inversely correlated with the number and function of nTreg lymphocytes. Rats exposed to arsenic also showed redistribution of nTreg cells and increased the levels of nTreg cells in spleen in experimental allergic encephalomyelitis model. The results indicated that arsenic seems to have a complex effect on nTreg cells [14].

### Immunological biomarkers for arsenic exposure in humans

Several biomarker studies have been performed to correlate arsenic exposure with human hazards. In these studies, immune functions, T-cell responses, and cytokine/chemokine dysregulation are revealed. Microarray genome-wide analysis from US New Hampshire residents revealed that overrepresentation of genes was involved in defense response, immune function, cell growth, apoptosis, cell cycle regulation, T-cell receptor (TCR) signaling pathway, and diabetes [15]. Killer cell immunoglobulin-like receptor and human major histocompatibility complex, Class II, HLA-DQ alpha 1, and perforin are among those with high correlations. Wu et al. [16], by DNA microarray and enzyme-linked immunosorbent assay, found that IL-1 beta, IL-6, CCL2, CXCL1, CXCL2, CD14, matrix metalloproteinase 1 were upregulated in persons with increased arsenic exposure. The association of CCL2 plasma protein level with blood arsenic remained significant after adjustment for other risk factors of cardiovascular diseases [16].

### Molecular mechanisms of defective T-cell activation and innate immunity by arsenic in humans

Although tremendous efforts have been aimed at determining the defect of cell immunity, the studies that tackle molecular mechanisms by which arsenic induces a decrease in T-cell proliferation, activation, and macrophage differentiation, and phagocytosis remains scanty. Arsenic compounds at very low concentrations enhanced DNA synthesis in phytohemagglutinin-stimulated proliferation of human lymphocytes, whereas higher concentrations inhibited DNA synthesis [17]. Arsenic induces aneuploidy [18,19], sister chromatid changes [20–22], and other chromosomal abnormalities [23] in lymphocytes. Ataxia telangiectasia mutated status confers sensitivity to arsenic cytotoxic effects through p53 [24]. Of note, among the metabolites of arsenic, MMA(III) and DMA(III) are candidate ultimate genotoxic forms of arsenic, and they are clastogens and not gene mutagens [25].

Arsenic interferes with the signaling transduction pathway of TCR activation by increasing basal and induced phosphorylation of lymphocyte-specific protein tyrosine kinase and Fyn (first kinases associated with TCR complex) in spleen cells [26]. Metabolites of arsenic can also interfere with cell division by means of tubulin disruption, leading to aneuploidy [27]. A major cellular source of methyl group, S-adenosyl-L-methionine, is able to reverse micronucleus formation induced by sodium arsenite and other cytoskeleton-disrupting agents in cultured human lymphocytes [28].

In contrast to the scanty studies that aim at the molecular mechanisms of arsenic on normal lymphocytes, there are a bunch of studies investigating the molecular mechanisms of arsenic-induced apoptosis in cells of lymphoma–leukemia. Arsenic remains the drug of choice for a special kind of acute myelocytic leukemia, acute promyelocytic leukemia. Arsenic induces normalization of differentiation of promyelocytes in acute promyelocytic leukemia. Phosphoinositide 3-kinase/Akt inhibition increases arsenic trioxide-induced apoptosis of acute promyelocytic and T-cell leukemia [29]. Arsenic induces apoptosis through activation of Bax in hematopoietic cells [30]. Furthermore, arsenic trioxide (As) and interferon-alpha synergize to induce cell cycle arrest and apoptosis that is modulated by bcl-2, bax, p53, and NF-kappaB [31,32]. Thus, the effects of arsenic on T-cell development may act as a double-sided sword and appear to be cell specific and concentration dependent.

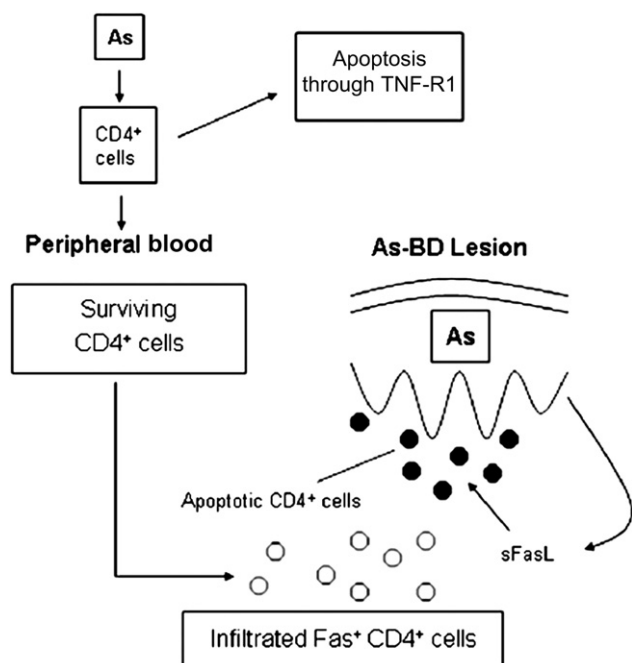
### Cell targets of immune system in arsenic-induced skin cancers

In humans, several immune cells could be the targets of arsenic. T cells are cells that have been studied extensively. Phytohemagglutinin-induced T-cell proliferation is impaired in T cells from patients with arsenic-induced Bowen's disease [33,34]. Indeed, there are dose-dependent responses of T cells to arsenic. Lower concentrations of arsenic (<1  $\mu\text{M}$ ) result in modest proliferations, whereas higher concentrations of arsenic (>2  $\mu\text{M}$ ) lead to apoptosis of T cells. Arsenic leads to preferentially CD4 cell apoptosis through an autocrine TNF-alpha loop that accentuates upregulation of TNF-alpha receptors on the surface and the increased release of TNF-alpha [33,35]. Not only the TNF-alpha loop is dysregulated but also the IL-2/IL-2R T-cell activation loop is impaired. Production of soluble IL-2 and expression of IL-2R on the T cells from patients with arsenic-induced Bowen's disease were significantly reduced, reflecting the decreased thymidine uptake in those T cells [36,37]. These results were confirmed in arsenic-exposed children from central Mexico [38] and adults in West Bengal [39], reemphasizing the impaired T-cell activation by arsenic.

Those patients also had impaired delayed-type hypersensitive response to 2,4-dinitrochlorobenzene. There were quantitative loss in numbers and qualitative impairment of dendrite formation in Langerhans cells located in the arsenic-induced Bowen's disease. It is evident that cell-mediated immunity was depressed in patients with arsenic-induced Bowen's disease [36].

## Selective CD4 cell apoptosis in the pathogenesis of arsenic-induced Bowen's disease

Both GM-CSF and TGF- $\alpha$  were found in the epidermis at clinically normal sites within 10 weeks after arsenic treatment *in vivo* and also from arsenic-treated keratinocytes [40,41]. Injection of neutralizing antibodies to GM-CSF after TPA application reduced the number of papillomas in TgAC mice that are prone to develop papillomas after arsenic exposure. These results suggested that arsenic enhances development of skin neoplasias by the chronic stimulation of keratinocyte-derived growth factors [40,41]. Although a clear link has been established for impaired T-cell proliferation by arsenic, the causative role of impaired T-cell activation and increased T-cell apoptosis with the cutaneous tumorigenesis has been seldom discussed. Patients with arsenic-induced Bowen's disease (As-BD) have lower percentage of peripheral CD4<sup>+</sup> cells compared with the control subjects; the CD4<sup>+</sup> cells from As-BD patients were less susceptible to arsenic-induced apoptosis because of reduced tumor necrosis factor receptor 1 expression. Systemically, the percentage of CD4<sup>+</sup> cells was decreased in the peripheral blood of As-BD patients. These residual CD4<sup>+</sup> cells were less susceptible to arsenic-induced apoptosis. However, once infiltrated into the As-BD lesions, fas ligand might mediate the selective CD4<sup>+</sup> cell apoptosis from keratinocytes (Fig. 2). This additional tumor-anti-immune phenomenon present in the cutaneous environment provides a reasonable explanation for the frequent occurrence of arsenical cancers in the skin [42].



**Figure 2.** A scheme of selective CD4 cell apoptosis in the pathogenesis of arsenic-induced Bowen's disease. As-BD = arsenic-induced Bowen's disease; sFasL = soluble Fas ligand; TNF-R1 = tumor necrosis factor receptor 1.

## Discussions

With millions of population worldwide still exposed to arsenic and the fact that arsenic is used to treat many forms of cancers, the investigations of mechanisms and effects of arsenic on immune system and tumor immunity, in particular, are of paramount importance. The indolent course of arsenic-induced skin cancer confers a good model to study tumor immunity, particularly chemical immunology, chemical carcinogenesis, and their interactions. Lessons from previous studies tell us that depending on its concentrations, arsenic exerts its modulatory effects on immune cells in terms of their proliferation, apoptosis, development, and differentiation. Through DNA damages, chromosome abnormalities, and signal transduction of survival and apoptosis pathways, T cells, CD4<sup>+</sup> cells, monocytes, and macrophages are all affected by arsenic. However, how those immune effects confer to arsenic carcinogenesis and tumor progression is seldom addressed. In our studies, we have shown that in arsenic-induced skin cancer, circulatory CD4<sup>+</sup> T cells undergo TNF- $\alpha$ -induced apoptosis; once they infiltrate into skin with arsenic-induced skin cancer, the infiltrated T cells undergo Fas–FasL-mediated apoptosis. Moreover, the question remains to determine what extent of the immune changes in Bowen's disease is caused by the effects of arsenic exposure rather than the consequences of the disease. From these previous literatures, the question cannot be easily answered. However, in the local skin, Fas–FasL interaction requires the participation of keratinocytes, the cell origin for arsenic-induced Bowen's disease. Perhaps, the recent breakthrough mice models about arsenic carcinogenesis might give some clues of immune regulations in the early development of arsenic cancers in the future.

Future research should incorporate comprehensive approaches to address the arsenic susceptibility in humans, the tumor immunity by arsenic, the prediction of immune biomarkers to the development of internal cancers, and the prognosis of arsenic-induced cancers. Many of those questions remain unanswered or largely unknown. The implementation of full-scale epidemiology studies, incorporating molecular immunology, cellular immunology, molecular epidemiology, high-throughput genetic and epigenetic profiling, and dedications will address the pathophysiology of adverse arsenic effects in humans, thus facilitating individualized treatments and prevention measures for exposed populations in the future. In chemical carcinogenesis, the attribute of chemical immunology should be always considered.

## Acknowledgments

This work was supported by grants from National Science Council (NSC95-2314-B-037-024, NSC96-2314-B-037-043, and NSC97-2320-B-010-013-MY3); National Health Research Institute (NHRI-EO-096-PP-11 and NHRI CN-PD-9611P); Kaohsiung Medical University (KMU) (KMU-QA096005); and KMU Center of Excellence for Environmental Medicine (98.2b).

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