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Inorganic arsenic alters expression of immune and stress response genes in activated primary human T lymphocytes

Corinne Martin-Chouly[a], Claudie Morzadec[a], Mélodie Bonvalet[a], Marie-Dominique Galibert[b], Olivier Fardel[a,c], Laurent Vernhet[a,*]

[a] Institut de Recherche en Santé Environnement Travail (IRSET), EA-4427 Signalisation et Réponses aux Agents Infectieux et Chimiques (SeRAIC), Université de Rennes 1, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France
[b] CNRS-UMR 6061, Laboratoire de Génétique et Développement, Equipe Régulation transcriptionnelle et oncogenèse, IFR140 GFAS, Université de Rennes-1, France
[c] Département HITC, Centre Hospitalier Universitaire (CHU) Rennes, 2 rue Henri Le Guilloux, 35033 Rennes, France

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ABSTRACT

Inorganic arsenic, a carcinogenic environmental contaminant, exerts immunosuppressive effects on human T lymphocytes. In particular, interleukin-2 (IL2) secretion and T cell proliferation are reduced when peripheral blood mononuclear cells (PBMC) from individuals chronically exposed to arsenic are stimulated *ex vivo* with lectins such as phytohemaglutinin (PHA). However, it is not clear whether the metalloid directly acts on T cells or blocks monocyte-dependent accessory signals activated by PHA. We report that *in vitro* pre-treatment of PBMC with sodium arsenite (NaAs) reduces IL2 secretion and T cell proliferation induced by PHA, but does not prevent expression of monocyte-derived cytokines (IL1, IL6, TNFα) functioning as lymphocyte-activating factors. In addition, we found that NaAs delays induction of IL2 and IL2 receptor α chain (IL2RA) mRNA levels in human primary isolated T cells activated by PHA. Kinetic analysis showed that NaAs pre-treatment first inhibits, but thereafter markedly increases, induction of IL2 and IL2RA mRNA when T cells are stimulated with PHA for 8 h and 72 h, respectively. We conducted whole genome microarray-based analysis of gene expression in primary T cell cultures derived from independent donors. NaAs systematically and significantly up-regulated a set of 35 genes, including several immune and stress genes, such as IL13, granulocyte-macrophage colony stimulating factor, lymphotoxin α and heme oxygenase-1 (HO-1). Up-regulation of HO-1, a stress and immunosuppressive protein, was rapidly detectable, both in T cells and in PBMC treated with NaAs. Inhibition of the immunosuppressive activity of HO-1 in PBMC however failed to prevent NaAs-dependent inhibition of T cell proliferation induced by PHA. Our findings demonstrate that, at least *in vitro*, inorganic arsenic acts directly on human T cells and impairs their activity, probably independently of HO-1 expression and monocyte-related accessory signals.

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1. Introduction

Inorganic arsenic (iAs) is a toxic compound found in the environment such that millions of individuals worldwide are chronically exposed (Ghosh et al., 2008). Long-term exposure to iAs is associated with increased incidence of several human diseases including cancer, atherosclerosis, diabetes and infection (Schuhmacher-Wolz et al., 2009; Rahman et al., 2009; Raqib et al., 2009). iAs alters the physiology of key immune cells and notably induces immunosuppressive effects that may contribute to its systemic toxicity (Selgrade, 2007; Raqib et al., 2009). Low concentrations of iAs significantly repress major functions in human macrophages and T lymphocytes (T cells), two immune cell types involved in the control of infection and tumor development (Lemarie et al., 2006a, 2008; Soto-Pena et al., 2006; Smyth et al., 2001).

We recently demonstrated that iAs blocks the differentiation of blood monocytes into functional macrophages by inhibiting survival signaling pathways (Lemarie et al., 2006a). In addition, at non-cytotoxic concentrations, the metalloid partially reverses the phenotypic and genotypic features of mature macrophages (Lemarie et al., 2006b; Bourdonnay et al., 2009a). iAs significantly reverses the expression of a large subset of genes specifically induced or repressed during the differentiation process, probably through an oxidative stress-related mechanism (Bourdonnay et al., 2009b). Similar functional and morphological effects are observed in macrophages obtained *ex vivo* by differentiation of blood monocytes taken from environmentally exposed individuals (Banerjee et al., 2009).

* Corresponding author. Tel.: +33 2 23 23 48 07; fax: +33 2 23 23 47 94.
E-mail address: laurent.vernhet@univ-rennes1.fr (L. Vernhet).

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Various studies have shown that iAs can also markedly alter development, activation and proliferation of T cells in addition to macrophages. *In vivo*, iAs treatment induces thymus atrophy in mice, probably through repression of genes involved in cell cycle progression, and reduces *ex vivo* proliferation of mitogen-stimulated splenocytes (Nohara et al., 2008; Soto-Pena and Vega, 2008). *Ex vivo* proliferation of peripheral blood T cells taken from environmentally exposed individuals is also significantly slower than that of those from non-exposed humans (Soto-Pena et al., 2006; Biswas et al., 2008). Similar results were observed in response to *in vitro* iAs treatment of human peripheral blood mononuclear cells (PBMC) stimulated with phytohemaglutinin (PHA) or concanavalin-A, two non-specific mitogenic lectins (Galicia et al., 2003; Gonsebatt et al., 1992). *In vitro* and *ex vivo*, iAs-dependent inhibition of blood peripheral T cell proliferation is generally associated with repression of interleukin-2 (IL2) secretion. This cytokine is central to the activation of T cells and their progression through the cell cycle (Robb et al., 1981). Mechanisms by which the metalloid deregulates T cell physiology, and notably IL2 expression, remain however largely unknown. PHA is thought to activate T cells via CD2 and T cell antigen receptors after binding to several membrane glycoproteins. Nevertheless, like stimulation with soluble CD3 antibody, full activation and proliferation of PHA-treated T cells requires an accessory signal, likely produced by monocytes. Once activated by PHA, T cells can physically interact with monocytes and stimulate secretion of monocyte-derived cytokines that are essential for optimal IL2 expression and T cell proliferation (Ceuppens et al., 1988; Jungo et al., 2001). Interleukin-1β (IL1β), interleukin-6 (IL6) and Tumor Necrosis factor α (TNFα) are the main cytokines secreted by monocytes allowing both maximal IL2 expression and PHA-stimulated T cell proliferation (Ceuppens et al., 1988; Hackett et al., 1988). In the absence of monocytes, PHA-treated T cells produce less IL-2 and do not proliferate.

Previous work on the effects of iAs on T cell activation used PBMC; consequently, these studies could not determine the cell type on which iAs acts. Indeed, iAs may either block monocyte-derived accessory signals or directly alter T cell activation. To discriminate between these possibilities, we investigated the effects of iAs on i) secretion of monocyte-derived cytokines that function as lymphocyte-activating factors in PBMC and ii) overall gene expression in activated primary cultures of human T cells.

We used sodium arsenite (NaAs), a main trivalent iAs form. It significantly reduced both the proliferation of human PHA-treated PBMC and IL2 secretion; however, it did not inhibit the expression of either IL6 or IL1 and TNFα, suggesting that it acts directly on T cells. Indeed, we found that NaAs substantially altered the expression of the IL2 and the IL2 receptor α chain (IL2RA) genes induced by PHA in human primary isolated T cells. In addition, DNA microarray experiments revealed that NaAs modulates the expression of several immune response and stress-related genes in these activated T cells.

2. Materials and methods

2.1. Chemicals

NaAs ($NaAsO_2$, Sigma–Aldrich) was dissolved in sterile distilled water to prepare stock solutions at 5 mM. PHA (Roche Diagnostics) was prepared in PBS at 1 mg/ml and stored at −20 °C. Tin protoporphyrin IX (SnPP, Alexis Biochemicals) was dissolved in DMSO.

2.2. Cell culture

PBMC were isolated from blood buffy coats of unrelated healthy donors by Ficoll gradient centrifugation. After separation of monocytes by a 1-h adhesion step, CD3+ T cells were purified from non-adherent cells by negative selection using the Dynabeads untouched human T cells (11344D) from Invitrogen. T cells were then cultured in RPMI medium supplemented with 10% fetal calf serum in the presence or absence of 1 μM NaAs for 2 h. The T cells were then treated or not treated with 1 μg/ml PHA for various times. The cytotoxic effects of NaAs on T cells were determined by trypan blue assays.

2.3. Cell proliferation assay

Proliferation of both human PBMC and isolated T cells was determined using the Cyquant NF Cell proliferation assay kit (Molecular Probes) as described in the experimental protocol for non-adherent cells. This assay involves measurement of fluorescent dye bound to the cellular DNA. Cells (5×10^5/ml) were seeded in 12-well plates, pre-treated or not with 1 μM NaAs, and cultured for 96 h in the presence or absence of PHA. The cells were then incubated for 30 min with the Cyquant NF DNA-binding dye and a plasma membrane permeabilization reagent. DNA fluorescence intensity, which is closely proportional to cell number, was quantified with a Gemini fluorescence microplate reader (Molecular Devices) using excitation at 485 nm and emission at 530 nm. Values for proliferation were calculated by subtracting mean fluorescence intensity of non-stimulated T cells, which are quiescent, from that of PHA-activated T cells pre-treated or not with NaAs.

2.4. Determination of cytokine levels

IL1β, IL2, IL6 and TNFα levels were assayed in supernatants of T cell cultures using Duoset ELISA development system kits (R&D systems) according to the manufacturer's instructions.

2.5. MTT assay

Metabolic activation of T cells was analyzed by measuring the activity of mitochondrial dehydrogenases using the 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, as previously described (Carmichael et al., 1987). Briefly, T cells were seeded in 24-well microplates, pre-treated or not with 1 μM NaAs, and then cultured in the presence or absence of PHA for 72 h. Each culture was then treated with 1 mg/ml MTT for an additional 3 h. The samples were centrifuged, the supernatant removed by aspiration and the blue formazan pellets were dissolved in dimethyl sulfoxide. Absorbance was then quantified at 540 nm using a Titertek Multiskan apparatus.

2.6. Flow cytometry

Membrane expression of IL2RA was determined by flow cytometry. Cells were incubated for 30 min in PBS with 5% human AB serum at 4 °C to avoid nonspecific antibody binding and then incubated with FITC-conjugated IgG1 immunoglobulin (isotype control) or with FITC-conjugated IL2RA monoclonal (IgG1) antibody for 20 min at 4 °C. Fluorescence related to immunolabeling was measured using a FC500 cytometer (Beckman Coulter, France).

2.7. RNA isolation and reverse-transcription real-time quantitative PCR (RT-qPCR) analysis

Following treatments and cell harvesting by centrifugation, total RNA was extracted from PBMC or T cells using the TRIzol method (Invitrogen Life Technologies). mRNAs were subjected to RT-qPCR analysis using the fluorescent SYBR Green methodology, gene-specific primers (QuantiTect Primer Assay, Qiagen) and an ABI Prism 7000 detector (Applied Biosystems), as previously described

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