APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2004; **18**: 431–437
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.690

Effects of arsenobetaine, a major organic arsenic compound in seafood, on the maturation and functions of human peripheral blood monocytes, macrophages and dendritic cells

Takami Ohta, Teruaki Sakurai* and Kitao Fujiwara

Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan

Received 14 October 2003; Revised 15 December 2003; Accepted 10 February 2004

We examine the *in vitro* immunotoxicity of synthetically pure arsenobetaine [AsBe; trimethyl (carboxymethyl) arsonium zwitterion], which is a major organic arsenic compound in seafood, on various human immune cells, such as peripheral blood monocytes, monocyte-derived macrophages and monocyte-derived dendritic cells (DCs). In particular, we examine the differentiation of monocytes into macrophages or DCs by comparing the effects of AsBe with those pentavalent inorganic arsenate. AsBe neither enhanced nor inhibited the differentiation of human monocytes into macrophages or DCs, and also did not affect their various immune functions. Furthermore, AsBe had no cytolethality in monocyte-derived macrophages or DCs even at a concentration of 5 mmol l^{-1}. In contrast, inorganic arsenate showed strong cytolethality in these human immune cells *in vitro* at micromolar concentrations. These data indicate that the organic arsenic compound AsBe in seafood has no *in vitro* immunotoxicity in human immune cells. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: arsenobetaine; arsenic; seafood; human; monocyte; differentiation; macrophage; dendritic cell; immunotoxicity; marine animal

INTRODUCTION

Arsenic has been considered to be a poison for a long time. Since the 1820s, it has been generally accepted as a potent environmental carcinogen for some human malignancies.[1] It has previously been reported that common marine animals, which are ingested daily as seafood in many countries, contain very high concentrations of arsenicals, ranging from about 4 to 80 μg g^{-1},[2] and that these arsenicals are commonly in the form of water-soluble organic arsenic compounds. The limit for arsenic in drinking water in Japan is 10 μg l^{-1}. If this limit was applied to seafood, as 10 ng g^{-1}, most of them would be deemed unfit for consumption, given that their contents are often 1000 times this concentration.[3] This finding has caused great concern with respect to the health of people who often ingest considerable amounts of seafood. In particular, it is very important for us to clarify the biological effects of the trimethyl (carboxymethyl) arsonium zwitterion, namely arsenobetaine (AsBe), which is a major organic arsenic compound in marine animals.[4] However, there have only been a few reports about them because sufficient amounts of pure AsBe for biological experiments have not been obtained.

In 1985, investigation of the acute toxicity of AsBe using synthetic pure material clarified that it had no acute toxicity in murine models even over 10 g kg^{-1} when it was administered orally.[5] Subsequently, we showed that AsBe had no immunotoxicity *in vitro* in murine immune effector cells, such as mouse peritoneal macrophages, alveolar macrophages and splenocytes,[6] and we also demonstrated that AsBe was not toxic *in vitro* to various mammalian cell lines, such as mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells, even over 20 mmol l^{-1}.[7] Furthermore, we recently reported that AsBe showed a unique biological effect on murine bone marrow (BM) cells *in vitro*; AsBe significantly enhanced the initial adhesion ability and viability of immature mouse BM

*Correspondence to: Teruaki Sakurai, Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan.
E-mail: sakurai@ls.toyaku.ac.jp

cells and subsequently increased the continuous survival of Mac-1 (CD11b) positive large spreading cells, especially granulocytes and macrophages, that originated from the BM cells.[8] These data imply that AsBe has a possible application to enhance some functions of immature immune cells. However, the reasons why AsBe enhances the survival of immature BM cells are not yet precisely clarified, and there are no data about the effects of AsBe on human immune cells.

Human peripheral blood monocytes are one of the immature immune effector cells and precursors of mature immune effector cells in the immune system. Monocytes can differentiate into macrophages by the granulocyte/macrophage colony stimulating factor (GM-CSF) or macrophage colony stimulating factor (M-CSF), and into dendritic cells (DCs) by the combination of GM-CSF plus interleukin-4 (IL-4).[9] Macrophages are one of the principal immune effector cells that play essential roles as secretory, phagocytic and antigen-presenting cells.[10] DCs are the major antigen-presenting cells capable of strongly stimulating primary T-cell responses.[9,11] In this study, we observed the effects of AsBe on human monocytes, monocyte-derived macrophages and monocyte-derived DCs, mainly concerning the differentiation of human monocytes into macrophages and/or DCs *in vitro*.

EXPERIMENTAL

Arsenic

AsBe was synthesized from trimethylarsine by reaction with ethyl β-bromo-propionate in an atmosphere of CO_2 (Tri Chemical Laboratory Inc., Yamanashi, Japan), and was twice recrystallized from acetone containing a trace of methanol.[5] It produced white prismatic crystals, with a melting point at 204 °C. Its structure was confirmed by ^1H nuclear magnetic resonance (NMR), ^{13}C NMR, high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS) and fast-atom bombardment MS. The purity of this synthesized AsBe was >99.9% as determined by thin-layer chromatography, HPLC–ICP-MS and gas chromatography–MS.[5–8] The synthesized AsBe was treated with Kurimover I (Kurita Water Industries Ltd, Tokyo, Japan), which is a removal agent for lipopolysaccharide (LPS), and LPS contamination of this AsBe was <0.000 04%(wt wt^{-1}) as determined by the endotoxin-specific limulus test. Sodium arsenate was purchased from Wako Pure Chemical Co. (Osaka, Japan), and was twice recrystallized from methanol. LPS contamination of this arsenate was <0.000 000 2%(wt wt^{-1}).

Human peripheral bloood monocytes

Human peripheral blood monocytes were obtained from normal healthy volunteers by centrifugation on a Lymphoprep (Nycomed, Oslo, Norway) gradient. Monocytes were separated with anti-CD14 monoclonal antibody (mAb)-coated MicroBeads using MACS single-use separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified monocytes were suspended in RPMI 1640 medium (Sigma Chemical Co., St Louis, MO, USA) containing 10% heat-inactivated fetal calf serum, 100 U l^{-1} penicillin, and 100 μg l^{-1} streptomycin (FCS-RPMI). Monocytes plated in flat-bottomed 96-well tissue culture plates (2×10^4 or 2×10^5 per well) or 12-well tissue culture plates (5×10^5 per well) were then cultured with 5000 U ml^{-1} recombinant human (rh) GM-CSF (PeproTech EC Ltd, London, UK), 1000 U ml^{-1} rhM-CSF (Genzyme Tech Co., Boston, MA, USA) or rhGM-CSF plus 2000 U ml^{-1} rhIL-4 (Genzyme) in the presence or absence of arsenic. Cultures were maintained in a humidified atomosphere of 5%CO_2–95% air at 37 °C. Cells which were incubated with rhGM-CSF or rhM-CSF for 6 days were differentiated into GM-type macrophages (GM-Mps) or M-type macrophages (M-Mps) respectively.[9,10] Cells which were incubated with rhGM-CSF plus rhIL-4 for 6 days were differentiated into DCs.[9,10]

Assay for cellular viability

The cellular viability was determined by measuring live cells using the AlamarBlue (AB) assay, which is similar to the methylthiazoletetrazolium assay. Briefly, cells (2×10^4 or 2×10^5/100 μl per well) were incubated on flat-bottomed 96-well tissue culture plates several times. At 3 h before the end of the incubation, 10 μl per well of AB solution (Iwaki Glass Co., Chiba, Japan) was added directly to the wells, and the absorbance at 570 nm (reference 600 nm) was measured using a microplate reader model 550 (Bio-Rad Laboratories, Hercules, CA, USA). Arsenic itself did not affect the absorbance of the AB solution, even at concentrations over 40 mmol l^{-1}.

Determination of the number and species of cells

Monocytes (2×10^4 or 2×10^5 per well) were incubated with various concentrations and combinations of rhGM-CSF, rhM-CSF, rhIL-4 or arsenic on flat-bottomed 96-well tissue culture plates for 6 days at 37 °C using FCS-RPMI. After the incubation, the number and the species of cells in the wells were determined under a microscope equipped with an eyepiece micrometer at ×200 magnification, whose microscopic field was equivalent to 0.25 mm^2. M-Mps are elongated and have a spindle-like morphology, and GM-Mps are round with a saucer-like morphology.[9,10] DCs induced by rhGM-CSF plus rhIL-4 displayed a dendritic morphology with delicate membrane projections that were veil-like or sheet-like processes.[9]

Phagocytosis assay

Functions of monocyte-derived macrophages were assessed by phagocytic ability using zymosan particles. Cells were incubated with zymosan (Sigma) in FCS-RPMI at a ratio of 40 particles per cell on flat-bottomed 96-well tissue culture plates for 30 min at 37 °C. After the incubation, the cells were stained using Diff-Quick stain kit (Kokusai Shiyaku Co., Hyogo, Japan), and the number of cells ingesting more

than three particles and phagocytosed particles per cell were determined by counting at least a total of 300 cells under the microscope. Phagocytic activity is expressed as the number of phagocytosed particles per cell which intook the particles.

Mixed leukocyte reactions

DC function was assessed by mixed leukocyte reactions (MLRs) assay. $CD14^-$ $CD4^+$ naive T cells were purified by negative selection of human blood peripheral monocytes using anti-CD14 mAb-coated MicroBeads and positive selection using anti-CD4 mAb-coated MicroBeads (Miltenyi Biotec). DCs or GM-Mps (stimulators; 5×10^3 per well) were incubated with allogeneic or autologous naive T cells (2×10^5 per well) on flat-bottomed 96-well tissue culture plates in 200 µl per well for 6 days at 37 °C; then, the proliferative response of T cells was determined by AB assay. Briefly, 6 h before the end of the incubation, 20 µl of AB solution was added directly to the 96-well plates, and the absorbance at 570 nm (reference 600 nm) was measured using a microplate reader model 550. Data are expressed as percentage absorbance using the values from T cells incubated without stimulators as 100%.

Assay for tumor necrosis factor α production

Monocytes (2×10^4 per well) were incubated with 1000 U ml^{-1} M-CSF or 5000 U ml^{-1} GM-CSF on 96-well tissue culture plates for 6 days at 37 °C. After the incubation, the cells were exposed to various concentrations of AsBe in the presence or absence of 1 ng ml^{-1} LPS (0111; B4; Sigma) for 2 days at 37 °C. The tumor necrosis factor α (TNFα) concentrations in the culture supernatants were quantitated by a double-sandwich enzyme-linked immunosorbent assay (ELISA). A 96-well plate was coated with mouse anti-human TNFα mAb (Genzyme) in phosphate-buffered saline (PBS). Uncoupled binding sites in the wells were blocked with PBS containing 1% bovine serum albumin, 5% sucrose, and 0.05% sodium azide. The wells were incubated with 50 µl of samples in triplicate for 40 min at 37 °C and then exposed to biotinylated goat anti-human TNFα polyclonal Ab (Genzyme). The plate was developed using a peroxidase-labeled streptavidin (Nichirei Co., Tokyo, Japan) and peroxidase substrate (TMB microwell peroxidase substrate system, Kirkegadd & Perry Lab., Gaithersburg, MD, USA). The absorbance at 450 nm (reference 630 nm) was measured using a microplate reader model 550 (Bio-Rad Laboratories). Aliquots of rhTNFα (Genzyme) were used to construct a standard curve, and the results were calculated as amounts of TNFα per microgram of cellular protein determined by BCA protein assay reagent (Piece Co., Rockford, IL, USA) with bovine serum albumin as the standard.

Arsenic analysis

Monocytes (1×10^6 per well) were incubated with 5 mmol ml^{-1} AsBe or arsenate on flat-bottomed 12-well tissue culture plates for 24 h at 37 °C. After the incubation, the cells were rinsed and lysed with 0.3 ml water. Then, 3 ml of nitric acid and 1 ml of sulfuric acid were added to the cell lysates and heated at 240 °C until dense fumes of sulfur trioxide appeared. The digested solutions were neutralized with ammonium hydroxide, followed by water to bring the solution volume up to 5 ml. 1 ml of hydrochloric acid, 0.5 ml of 20% ascorbic acid and 0.5 ml of 20% potassium iodide were then added to the solutions. The total arsenic amount was analyzed by hydride generation coupled with atomic absorption spectrometry using SpestraAA-220 (Varian Australia Pty Ltd., Mulgrave Victria, Australia). The results are expressed as the cellular arsenic content per gram cellular proteins determined by BCA protein assay.[2,12,13]

Statistics

Statistical evaluations in experiments were expressed as the arithmetic mean plus/minus the standard error of the mean (SEM) and performed by analysis of variance followed by Dunnett's multiple comparison test or Tukey's honestly significant difference test. A value of $p < 0.05$ was considered significant in all cases.

RESULTS

Effect of AsBe on the differentiation into macrophages from human monocytes

M-CSF and GM-CSF are potent cytokines which induce differentiation and proliferation of monocytes into macrophages. Human monocytes can differentiate into two morphologically distinct types of macrophage, i.e. M-Mp and GM-Mp, by M-CSF and GM-CSF respectively. We examined the effects of a pentavalent organic arsenic AsBe on these maturations from monocytes into macrophages, comparing it with the effects of a pentavalent inorganic arsenic arsenate. Monocytes were incubated with M-CSF or GM-CSF in the presence or absence of various concentrations of AsBe or arsenate for 6 days at 37 °C; then, the numbers of monocyte-derived macrophages were counted. As shown in Fig. 1A and B, AsBe did not affect the number of monocyte-derived M-Mps and GM-Mps even at a concentration of 5 mmol l^{-1}, although arsenate significantly decreased it; its inhibitory concentration *in vitro* in 50% of a population (IC_{50}) was 280 µmol l^{-1} or 180 µmol l^{-1} in M-Mps or GM-Mps respectively. In morphological investigations, AsBe also did not affect the morphological changes in either type of monocyte-derived macrophage. Additionally, we investigated the cellular viability of monocyte-derived macrophages exposed to arsenic during their differentiations by the AB assay. As shown in Fig. 1C and D, AsBe had no effect on their viability, although arsenate showed strong cytolethality on them; its lethal concentration *in vitro* in 50% of a population (LC_{50}) was 200 µmol l^{-1}, and most cells were killed by millimolar concentrations of arsenate.

Phagocytic ability is one of the principal immune functions of macrophages.[10] Table 1 shows the phagocytic ability of monocyte-derived macrophages exposed to AsBe during their differentiations. Monocytes were incubated with M-CSF or

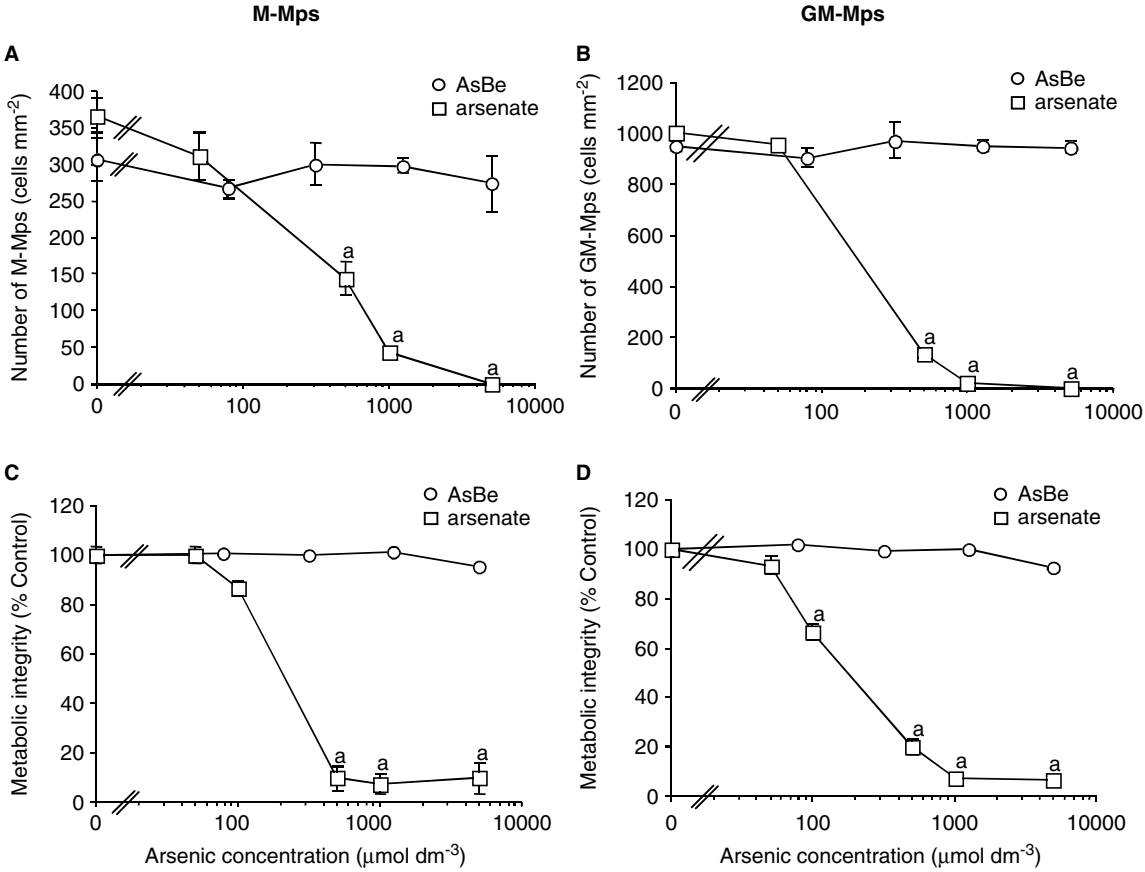

Figure 1. Effect of arsenic on the differentiation into macrophages from monocytes. Monocytes (2×10^4 per well) were incubated with M-CSF (A, C) or GM-CSF (B, D) in the presence or absence of various concentrations of AsBe (○), arsenate (□) or medium alone on 96-well tissue culture plates for 6 days at 37 °C. (A, B) The number and species of monocyte-derived macrophages (A, M-Mps; B, GM-Mps) were counted. (C, D) The cellular viability of monocyte-derived macrophages (C, M-Mps; D, GM-Mps) was determined by AB assay. One representative experiment out of five similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $p < 0.001$ comparison with monocytes incubated without arsenic.

Table 1. Effect of AsBe on the phagocytic activity of monocyte-derived macrophages[a]

Cytokine	AsBe (5 mmol l^{-1})	Percentage of phagocytes	Phagocytic activity[b]
M-CSF	−	71.2 ± 1.5	4.84 ± 0.33
	+	74.2 ± 7.6	5.22 ± 0.53
GM-CSF	−	76.3 ± 5.3	6.37 ± 0.63
	+	69.4 ± 3.1	7.28 ± 0.23

[a] Monocytes were incubated with M-CSF or GM-CSF in the presence or absence of AsBe (5 mmol l^{-1}) for 6 days at 37 °C. After the incubation, monocyte-derived macrophages were further incubated with zymosan at a ratio of 40 particles per cell for 30 min at 37 °C. The numbers of cells ingesting more than three particles and phagocytosed particles were determined by counting at least 300 cells under a microscope. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.
[b] Phagocytic activity is the number of phagocytosed particles per cell which intook the particles.

GM-CSF in the presence or absence of 5 mmol l^{-1} AsBe for 6 days at 37 °C. The phagocytic activity of the cells was then assessed, and AsBe was found to have no effect on the phagocytic ability of these macrophages.

Effect of AsBe on the differentiation into DCs from human monocytes

Using the combination of GM-CSF and IL-4, human monocytes can differentiate into DCs, which are the major antigen-presenting cells capable of stimulating primary T-cells responses.[9,11] We examined the effects of AsBe on this maturation from monocytes into DCs by comparison with the effects of arsenate. Monocytes were incubated with GM-CSF plus IL-4 in the presence or absence of various concentrations of AsBe or arsenate for 6 days at 37 °C; then, the number of monocyte-derived DCs were counted. As shown in Fig. 2A, AsBe did not affect the number of monocyte-derived DCs even at a concentration of 5 mmol l^{-1}, although arsenate significantly decreased it; its IC_{50} value was 590 μmol l^{-1}.

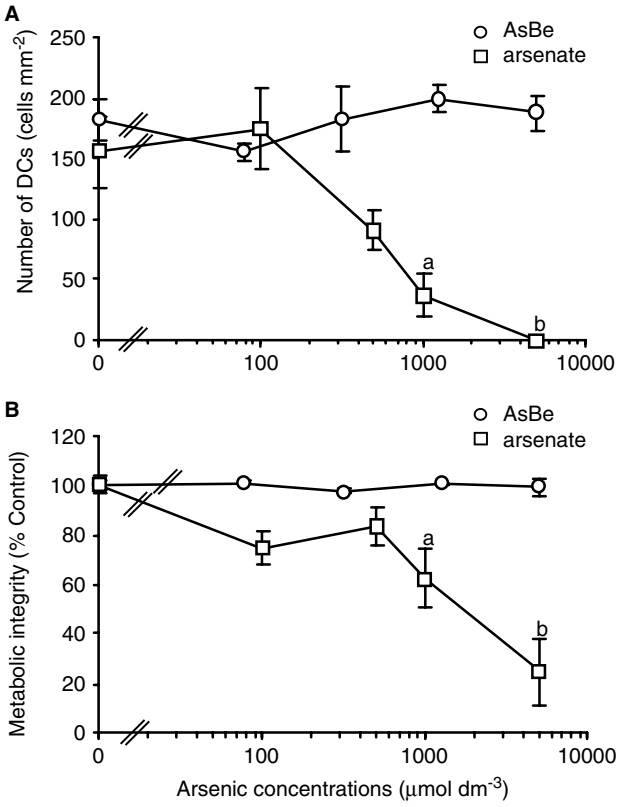

Figure 2. Effect of arsenic on the differentiation into DCs from monocytes. Monocytes (2×10^4 per well) were incubated with GM-CSF plus IL-4 in the presence or absence of various concentrations of AsBe (○), arsenate (□) or medium alone on 96-well tissue culture plates for 6 days at 37 °C. (A) The numbers of monocyte-derived DCs were counted. (B) The cellular viability of monocyte-derived DCs was determined by AB assay. One representative experiment out of five similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $p < 0.05$ comparison with monocytes incubated without arsenic. [b] $p < 0.01$.

When the cellular viability of monocyte-derived DCs exposed to AsBe during their differentiations was determined by the AB assay, AsBe also had no effect on the cellular viability, although arsenate showed significant cytolethality with a LC_{50} value of 1.6 mmol l^{-1}, and most cells were killed by millimolar concentrations of arsenate (Fig. 2B).

The most important characteristic of DCs is their strong T-cell stimulating activity.[9] DCs, but not GM-Mps, stimulate both allogeneic and autologous T cells in MLRs.[9,14] Table 2 shows the effect of AsBe on the T-cell stimulating activity (MLRs) of monocyte-derived DCs exposed to AsBe during their differentiations. Monocytes were incubated with GM-CSF plus IL-4 in the presence or absence of 5 mmol l^{-1} AsBe for 6 days at 37 °C; the T-cell stimulating activity of these cells was then determined by MLRs assay. As a result, monocyte-derived DCs, but not GM-Mps (negative control), strongly stimulated the T-cell responses, and the addition of AsBe during the differentiation of monocytes into DCs did not affect this immune function of DCs.

Table 2. Effect of AsBe on MLRs of monocyte-derived DC[a]

Stimulator	AsBe (5 mmol l^{-1})	MLR[b] (% control) Allogeneic	Autologous
GM-Mps	−	115.6 ± 4.4	68.5 ± 8.5
DCs	−	193.9 ± 7.9[c]	142.8 ± 16.1[d]
	+	219.1 ± 8.4[c]	124.2 ± 1.3[e]

[a] Monocytes were incubated with GM-CSF or GM-CSF plus IL-4 in the presence or absence of AsBe (5 mmol l^{-1}) for 6 days at 37 °C. After the incubation, the cells (stimulators; GM-Mps or DCs) were further incubated with allogeneic or autologous T cells for 6 days at 37 °C. The proliferative response of the T cells was then determined by AB assay. One representative experiment out of four similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.
[b] Data are expressed as percentage absorbance using the values from T cells incubated without stimulators as 100%.
[c] $p < 0.001$, comparison with T cells incubated with GM-Mps as a stimulator.
[d] $p < 0.01$.
[e] $p < 0.05$.

Effect of AsBe on the viability and functions of human monocyte-derived macrophages and DCs

We examined the cytotoxic effects of AsBe using human mature immune effector cells, such as human monocyte-derived macrophages and DCs, by comparison with the effects of arsenate. Monocyte-derived M-Mps, GM-Mps or DCs were incubated with various concentrations of AsBe or arsenate for 2 days at 37 °C; the cellular viability was then determined by the AB assay. As shown in Fig. 3, AsBe had no cytolethality on these mature immune effector cells, even at a concentration of 5 mmol l^{-1}, although arsenate showed significant cytolethality in these cells with LC_{50} values of 850 μmol l^{-1}–1.9 mmol l^{-1}, and most cells were killed by millimolar concentrations of arsenate.

Additionally, we investigated the effects of AsBe on the functions of mature immune effector cells. Macrophages are known to be very sensitive to changes in environmental conditions and release various immune inflammatory factors when they are stimulated.[12,15,16] Figure 4 shows the effect of AsBe on the TNFα secretion, which is one of the potent inflammatory cytokines,[12,15] in monocyte-derived macrophages. M-Mps and GM-Mps were incubated with various doses of AsBe in the presence or absence of 1 ng ml^{-1} LPS for 2 days at 37 °C; the concentrations of TNFα in the culture supernatants were measured by double-sandwich ELISA. As a result, AsBe had no effect on the release of TNFα when the macrophages were stimulated by LPS. AsBe alone (without LPS) also did not induce the release of TNFα from macrophages (data not shown).

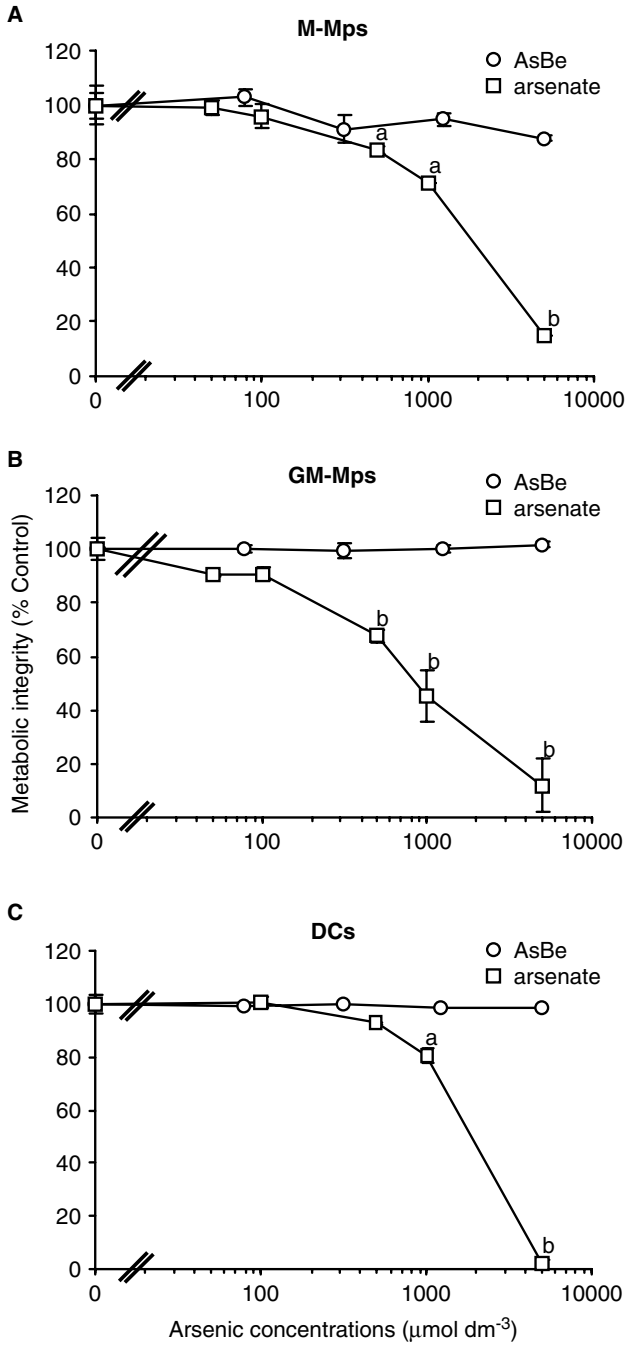

Figure 3. Cytolethality of arsenic on the monocyte-derived macrophages and DCs. Monocytes (2×10^4 per well) were incubated with M-CSF (A), GM-CSF (B) or GM-CSF plus IL-4 (C) on 96-well tissue culture plates for 6 days at 37 °C. After the incubation, the cells were further exposed to various concentrations of AsBe (○), arsenate (□) or medium alone for more 2 days at 37 °C. The cellular viability of the cells (A, M-Mps; B, GM-Mps; C, DCs) was determined by AB assay. One representative experiment out of five similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $p < 0.01$ comparison with control cells incubated with medium alone. [b] $p < 0.001$.

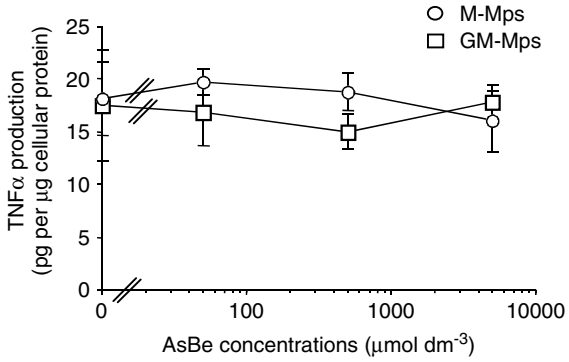

Figure 4. Effect of AsBe on TNFα production from monocyte-derived macrophages. Monocytes (2×10^4 per well) were incubated with M-CSF (○) or GM-CSF (□) on 96-well tissue culture plates for 6 days at 37 °C. After the incubation, the cells were further exposed to various concentrations of AsBe in the presence of 1 ng ml^{-1} LPS for 2 days at 37 °C. TNFα concentrations in the culture supernatants of monocyte-derived macrophages (○, M-Mps; □, GM-Mps) were measured by ELISA. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.

Furthermore, we investigated the effects of AsBe on the phagocytic ability of monocyte-derived macrophages and on the T-cell stimulating ability of monocyte-derived DCs. Macrophages or DCs differentiated from monocytes were incubated with or without 5 mmol l^{-1} AsBe for 2 days at 37 °C; the phagocytic activity of the macrophages and MLRs of the DCs were then assessed. The results show that AsBe had no effect on either phagocytic activity or T-cell stimulating activity of these mature immune effector cells (data not shown).

Cellular arsenic uptake in human monocytes

Monocytes (1×10^6 per well) were incubated with 5 mmol l^{-1} AsBe or arsenate for 24 h, and the arsenic amount in the cell lysates was determined by hydride generation coupled with atomic absorption spectrometry. As a result, the cellular uptake of AsBe in human monocytes was about 10-fold lower than that of arsenate; when monocytes were exposed to arsenate, 4106 ± 77.0 μg of arsenic per gram cellular protein was detected from the cell lysates, but only 456.3 ± 97.8 μg of arsenic per gram cellular protein was detected when monocytes were incubated with AsBe.

DISCUSSION

Marine animals, containing very high concentrations of arsenicals,[2–4] are ingested daily as seafood in many countries in the world. These arsenicals are commonly in the form of water-soluble organic arsenic compounds metabolized

by marine animals from inorganic arsenic. In the marine ecosystem, it has been demonstrated that inorganic arsenic in sea water is probably taken up into seaweed and metabolically methylated to dimethylarsinoyl ribosides (arsenosugars).[17] Arsenosugars are further converted into trimethyl arsenic compounds in many species of marine animal. Most of the trimethyl arsenic contained in marine animals is AsBe,[4] and AsBe is thought to be the final metabolite in the arsenic cycle of marine ecosystems because it is widely distributed in various species of marine animal. Many people take in considerable amounts of AsBe through ingesting seafood. Thus, it is very important for us to investigate the biological effects of AsBe on the human living system. However, there have been only a few reports about those biological effects. In this study, we examined whether AsBe has any toxicological and/or biological effects on human immune effector cells, such as human peripheral blood monocytes, macrophages and DCs, which are known to be very sensitive to changes in environmental conditions.[12,15,16,18]

One of the principal characteristics of monocytes is to differentiate into some mature immune effector cells, such as macrophages and DCs.[9,10] In this study, we investigated the effects of AsBe on the differentiation of monocytes into macrophages or DCs *in vitro* and compared this with the effects of inorganic arsenate. As shown in Figs 1 and 2, AsBe had no effect on the number and cellular viabilities of monocyte-derived macrophages or DCs, although arsenate showed significant cytolethality in them at micromolar concentrations. As shown in Tables 1 and 2, AsBe did not affect the principal functions of monocyte-derived macrophages or DCs. Laupeze *et al*.[19] reported that polycyclic aromatic hydrocarbons, known to be environmental carcinogens, such as benzo(a)pyrene (BP), had immunotoxicity on differentiation and maturation of monocyte-derived DCs; 10 μmol l^{-1} BP exposure during monocyte differentiation into DCs by GM-CSF plus IL-4 markedly decreased endocytic activity and T-cell stimulating activity. These data imply that the experimental model using differentiation of monocytes into DCs is capable of evaluating the immunotoxicities of lower concentrations of chemicals having no cytolethality. Thus, this assay, using differentiation of monocytes into macrophages or DCs, is believed to be more sensitive for evaluating chemical cytolethality than an assay using simple cytolethality. It is suggested that AsBe had no toxicological effects on the human living system.

The reasons why AsBe shows no cytolethality in human immune cells has not yet been clarified. However, we demonstrated in this study that the cellular uptake of a pentavalent organic arsenic, AsBe, was markedly lower than that of a toxic pentavalent inorganic arsenic, i.e. arsenate. Thus, hereafter, it is necessary to investigate the relationship between the cellular uptake and the chemical forms of arsenic compounds.

In 2001 we found that AsBe significantly enhanced the initial adhesion ability and viability of immature mouse BM cells and subsequently increased the continuous survival of Mac-1 (CD11b) positive large spreading cells, especially granulocytes and macrophages, which originated from the BM cells.[8] This implied that AsBe had a possible application as a biological response modifier (BRM) to increase the number of granulocytes and macrophages. We previously reported that AsBe had no acute *in vitro* cytolethality in mouse macrophages, mouse lymphocytes, rat liver cells and human skin cells.[6–8,20,21] The present study shows that AsBe neither enhanced nor inhibited the differentiation of human monocytes into macrophages or DCs and did not disrupt the major functions of monocyte-derived macrophages or DCs exposed during their differentiations. Taken together, it may be possible to use AsBe as a BRM without fatal toxic side effects.

In summary, we have demonstrated that AsBe was not very cytotoxic in human monocytes, monocyte-derived macrophages and DCs. AsBe is the majority component of the arsenic contained in marine animals that are ingested daily as seafood in many countries. Thus, the present study has significance in food hygiene.

Acknowledgements

We express our thanks to Dr Masumi H. Sakurai (Azabu University, Japan) for the limulus test and help with the preparation of this manuscript, and to Mr Norihumi Tomita and Miss Yukie Hariya for their excellent technical assistance.

REFERENCES

1. Morton WE, Dunnette DA. In *Arsenic in the Environment Part II: Human Health and Ecosystem Effects*, Nriagu JO (ed.). John Wiley and Sons: New York, 1994; 17–34.
2. Kaise T, Hanaoka K, Tagawa S, Hirayama T, Fukui S. *Appl. Organometal. Chem.* 1988; **2**: 539.
3. Le X-C, Cullen WR, Reimer KJ. *Clin. Chem.* 1994; **40**: 617.
4. Edmonds JS, Francesconi KA, Cannon JR, Raston CR, Skelton BW, White AH. *Tetrahedron Lett.* 1977; **18**: 1543.
5. Kaise T, Watanabe S, Itoh K. *Chemosphere* 1985; **14**: 1327.
6. Sakurai T, Kaise T, Matsubara C. *Appl. Organometal. Chem.* 1996; **10**: 727.
7. Kojima C, Sakurai T, Ochiai M, Kumata H, Qu W, Maalkes MP, Fujiwara K. *Appl. Organometal. Chem.* 2002; **16**: 421.
8. Sakurai T, Fujiwara K. *Br. J. Pharmacol.* 2001; **132**: 143.
9. Akagawa KS, Takasuka N, Nozaki Y, Komuro I, Azuma M, Ueda M, Naito M, Takahashi K. *Blood* 1996; **88**: 4029.
10. Akagawa KS. *Int. J. Hematol.* 2002; **76**: 27.
11. Banchereau J, Steinman RM. *Nature* 1998; **392**: 245.
12. Sakurai T, Kaise T, Matsubara C. *Chem. Res. Toxicol.* 1998; **11**: 273.
13. Sakurai T, Qu W, Sakurai MH, Waalkes MP. *Chem. Res. Toxicol.* 2002; **15**: 629.
14. Thomas R, Davis LS, Lipsky PE. *J. Immunol.* 1993; **151**: 6840.
15. Mosser DM. *J. Leukoc. Biol.* 2003; **73**: 209.
16. Issekutz AC, Issekutz TB. *J. Immunol.* 1993; **151**: 2105.
17. Edmonds JS, Francesconi KA. *Nature* 1981; **289**: 602.
18. Manome H, Aiba S, Tagami H. *Immunology* 1999; **98**: 481.
19. Laupeze B, Amiot L, Sparfel L, Ferrec EL, Fauchet R, Fardel O. *J. Immunol.* 2002; **168**: 2652.
20. Sakurai T. *Appl. Organometal. Chem.* 2002; **16**: 401.
21. Sakurai T, Kojima C, Ochiai M, Ohta T, Fujiwara K. *Int. Immunopharmacol.* 2004; **4**: 179.