Exposure to Moderate Arsenic Concentrations Increases Atherosclerosis in ApoE$^{-/-}$ Mouse Model

Maryse Lemaire,*† Catherine A. Lemarié,† Manuel Flores Molina,*† Ernesto L. Schiffrin,† Stéphanie Lehoux,† and Koren K. Mann*†,1

*Department of Oncology and †Department of Medicine, Lady Davis Institute for Medical Research, McGill University, Montreal, H3T 1E2, Canada

1To whom correspondence should be addressed at Lady Davis Institute for Medical Research, McGill University, 3755 Cote Ste Catherine Road, Montre´al, QC, H3T 1E2, Canada. Fax: +514-340-8717. E-mail: koren.mann@mcgill.ca.

Received February 16, 2011; accepted April 15, 2011

Arsenic is a widespread environmental contaminant to which millions of people are exposed worldwide. Exposure to arsenic is epidemiologically linked to increased cardiovascular disease, such as atherosclerosis. However, the effects of moderate concentrations of arsenic on atherosclerosis formation are unknown. Therefore, we utilized an in vivo ApoE$^{-/-}$ mouse model to assess the effects of chronic moderate exposure to arsenic on plaque formation and composition in order to facilitate mechanistic investigations. Mice exposed to 200 ppb arsenic developed atherosclerotic lesions, a lower exposure than previously reported. In addition, arsenic modified the plaque content, rendering them potentially less stable and consequently, potentially more dangerous. Moreover, we observed that the lower exposure concentration was more atherogenic than the higher concentration. Arsenic-enhanced lesions correlated with several proatherogenic molecular changes, including decreased liver X receptor (LXR) target gene expression and increased proinflammatory cytokines. Significantly, our observations suggest that chronic moderate arsenic exposure may be a greater cardiovascular health risk than previously anticipated.

Key Words: arsenic; atherosclerosis; environmental exposure; plaque composition; cholesterol.

Arsenic is a widespread environmental drinking water contaminant (IGRAC; Nordstrom, 2002). The World Health Organization (WHO, 2008) recommends that the arsenic level in municipal water not exceed 10 ppb. Despite this limit, well water in many North American counties averages 9-fold higher than 10 ppb (Engel and Smith, 1994; Frost et al., 2003). Particularly high endemic levels (up to 2.5 ppm) are found in Bangladesh, the southwestern United States, and Taiwan (Nordstrom, 2002). Epidemiologic evidence indicates that humans exposed to arsenic have an increased risk not only of cancer (Abernathy et al., 2003) but also of developing cardiovascular diseases, such as ischemic heart disease and atherosclerosis (Chen et al., 1996; Engel and Smith, 1994; Medrano et al., 2010; Navas-Acien et al., 2005; Tseng, 2008; Wang et al., 2002). In some cases, relatively low concentrations of arsenic have even been associated with cardiovascular mortality (Argos et al., 2010; Engel and Smith, 1994; Medrano et al., 2010).

Despite the clear epidemiologic links between arsenic and an increased risk of atherosclerosis, the mechanisms by which arsenic enhances atherosclerosis are unclear. Inflammation and lipid accumulation are key components of atherosclerosis, both of which may be proatherogenic effects of arsenic. Arsenic is reported to increase the expression of various inflammatory molecules (Bunderson et al., 2004; Srivastava et al., 2009; Straub et al., 2009). In addition, we have previously shown that in vitro arsenic inhibits key regulators of lipid homeostasis, the liver X receptors (LXRs) (Padovani et al., 2010). LXRs are lipid-sensing receptors, which bind oxysterols derived from cholesterol metabolism and regulate genes that control adequate cholesterol clearance from the macrophage (Baranowski, 2008). In part because they stimulate cholesterol efflux, LXR ligands are considered antiatherogenic. Any alteration in the properties of the activated macrophages within the arterial walls, including modifications in their inflammatory profile or in their reverse cholesterol transport capacities, has the potential to enhance lesion formation.

Mouse models of atherosclerosis confirm human epidemiological reports and show that arsenic can induce and accelerate plaque formation (Simeonova et al., 2003; Srivastava et al., 2009). ApoE$^{-/-}$ mice are well-described models of atherosclerosis where cholesterol accumulates within macrophages and the liver cannot clear plasma lipoproteins. They also produce plaques with similar histopathological characteristics to those found in humans (Breslow, 1996). In this model, both early postnatal and adult exposures to 49 ppm arsenic increased atherosclerosis (Srivastava et al., 2009), whereas 10 ppm arsenic exacerbated the atherosclerotic phenotype in ApoE$^{-/-}$ mice with low-density lipoprotein receptor deletion (Bunderson et al., 2004). However, in all these previous reports, particularly high arsenic concentrations were evaluated, which model only...
those populations exposed to particularly high arsenic concentrations.

The maximum contaminant level has been defined using primarily risk analysis of the carcinogenic effects of arsenic, not its cardiovascular consequences. Recent studies show that lower arsenic exposures are sufficient to affect the cardiovascular system than to those that will cause cancer (Padovani et al., 2010; Soucy et al., 2005; Straub et al., 2007). For instance, in mice, a low arsenic exposure (5–250 ppb for 5 weeks) is enough to change vascularization and trigger angiogenic gene expression in cardiac tissue (Soucy et al., 2005). In humans, the minimal dose at which arsenic exposes subjects to risk of developing atherosclerosis remains largely undefined because only a few reports have studied the effects of low exposure arsenic on human cardiovascular health. However, these published data showed an increased risk of cardiovascular disease with exposure to low environmental concentrations of arsenic (Engel and Smith, 1994; Medrano et al., 2010; Zierold et al., 2004). Therefore, we investigated the effect of moderate concentrations of arsenic on the development of atherosclerosis. We exposed ApoE<sup>−/−</sup> mice to arsenic for either 8 or 13 weeks. Interestingly, we observed more extensive atherosclerotic lesions in the group exposed to the lower concentration of arsenic (200 ppb) when compared with either the control mice or those exposed to the moderate concentration of 1000 ppb arsenic. To our knowledge, 1000 ppb is the lowest concentration of arsenic previously reported to exacerbate plaque formation (Srivastava et al., 2009). Furthermore, we observed that arsenic exposure modifies plaque content toward a less stable phenotype (Shah, 2003). Significantly, our data show that arsenic enhances atherosclerotic lesion development at lower exposure than previously reported and thus, may be a greater cardiovascular health risk than previously anticipated.

**MATERIALS AND METHODS**

**Animals housing and exposure protocol.** B6.129P2-Apoetm1Unc/J (ApoE<sup>−/−</sup>) male mice were obtained from Jackson laboratory (Bar Harbor, ME). Mice were acclimated to housing conditions for at least 2 weeks before experiments. They received food and water ad libitum. Prior to arsenic exposure, all mice were maintained on a normal chow diet and tap water. The McGill animal use committee approved the experimental protocol, and animals were handled in accordance with institutional guidelines.

For experiments, ApoE<sup>−/−</sup> male mice (5 weeks old, n ≥ 5 animals per group) were either maintained on tap water or on tap water-containing sodium arsenite (NaAsO<sub>2</sub>) (Sigma, MO). Mice that received NaAsO<sub>2</sub> were exposed either to 1000 ppb (1.73 mg/l NaAsO<sub>2</sub>) or 200 ppb (0.35 mg/l NaAsO<sub>2</sub>) for either 8 or 13 weeks. Solutions containing arsenic were refreshed every 2–3 days to minimize oxidation. The mice were either fed with normal chow or high-fat diet (2018 and TD 94059, respectively; Harlan Laboratories Inc., WI). The normal chow contained 5% fat (by weight) with no cholesterol, whereas the atherogenic high-fat diet contained 15.8% fat (half from cocoa butter) including 1.25% cholesterol. Minimal levels of arsenic were detected by inductively coupled plasma mass spectrometry (McGill Geochemistry department) in the tap water (0.75 ppb), in the regular chow (1.90 ppb), and in the high-fat diet (1.50 ppb) ± SD of 5%, with a the detection limit is 0.65 ppb.

**Plasma analyses.** Blood (0.6 ml) was removed by cardiac puncture and was collected in tubes coated with micronized silica particles to accelerate clotting and favor serum separation from cells and fibrin (BD Vacutainer SST). Plasma cholesterol, high-density lipoprotein (HDL), and liver enzyme (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) activities were determined by the Jewish General Hospital core laboratories (n ≥ 5 animals per group). Plasma triglyceride levels were assessed by the Charles River Research Animal Diagnostic Services (MA) (n = 3 animals per group). For cytokine measurements, blood was collected in EDTA-coated tubes (Sarstedt, Germany) and the cytokine levels (macrophage inflammatory protein-1 [MIP1α] and interferon-γ [IFNγ]) were measured in the plasma using an immunoassay kit (multiplex bead-based) on a Bio-Plex 200 (Bio-Rad Laboratories, ON, Canada). Each sample (n ≥ 4 animals per group) was analyzed in duplicate (technical replicate).

**Atherosclerotic lesion characterization.** The entire aorta, from the heart to the iliac arteries, was removed and rinsed with PBS and fixed in 4% paraformaldehyde. Periadventitial tissue was removed and the aorta was cut longitudinally. The aortic surface was stained en face with oil red O (Electronic Microscopy Sciences, PA). Lipid staining is not necessary for quantification of atherosclerotic lesions, but it can be useful in assessing the disease when lesions are small (Daugherty and Whitman, 2003). Percentage of lesion area of the aortic arch, as defined as the region from ascending arch to the first intercostal arteries, was evaluated with the Infinity Analyze software 5.0 (Lumenera, Canada). The heart was removed, rinsed, fixed in 4% paraformaldehyde and incubated overnight in a 30% sucrose solution (Braun et al., 2003). The tissues were frozen then in Tissue Tek OCT (Sakura, CA) reagent, and serial cryosections of 6-μm thickness were cut from the origin of the aortic root throughout the aortic sinus. Five to seven sections per animal were stained with oil red O and the mean lesion area was calculated using ImageJ software (National Institute of Health). Percentage of lesion area was evaluated relative to the total aortic sinus area. The lipid content and the collagen content of the plaque were evaluated with ImageJ, using their specific stains (oil red O and picrosirius red [Polysciences, PA], respectively).

**Immunofluorescence analyses.** Smooth muscle cell and macrophage content within the entire plaque area of the aortic sinus were determined by immunofluorescence using monoclonal anti-α-smooth muscle cell actin (clone 1A4) and mona-2, respectively (Abcam, MA). Briefly, the sections were rinsed and blocked with 3% bovine albumin serum (Sigma). The aortic sinus sections were incubated then with primary antibody (1:100), rinsed, and further incubated with fluorescently labeled secondary antibodies (1:500) (Invitrogen). At least five sections per animal were stained. Images were acquired using Infinity Capture software and camera and analyzed using ImageJ software. The presence of the immunofluorescent marker was quantified and expressed in percentage of the total lesion area.

**Thioglycollate-elicited macrophages.** In order to obtain sufficient in vivo macrophages, 1.5 ml of 4% thioglycollate solution (Criterion, CA) was injected ip in mice exposed to tap water or arsenic. Seventy-two hours later, the ascitic fluid containing elicited macrophages was collected (Padovani et al., 2010). Gene expression analysis was then performed on those cells (n = 4 animals per group).

**Analysis of gene expression by real-time PCR.** Total RNA from elicited macrophages was isolated using TRIzol reagent (Invitrogen, ON, Canada), followed by chloroform extraction and nucleic acid precipitation in isopropanol. RNA was quantitated on a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, DE). cDNA was synthesized from 5 μg RNA by using random primers and Superscript II reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed using 7500 Fast PCR (Applied Biosystems, CA) under standard conditions of 60°C annealing temperature for 40 cycles. All primers were designed using Primer Express 2 Software. SYBR
green chemistry was used with specific primers (listed in Supplementary Table 1) for all genes. Results were analyzed using the ΔΔCt method and control-treated animals as calibrator samples. The analyzed genes were expressed relative to the murine acidic ribosomal phosphoprotein P0 (36B4) housekeeping gene.

**Statistical considerations.** For statistical analysis, the one-way ANOVA was performed and the p value was evaluated with a Tukey’s post hoc test using the GraphPad Instat software (San Diego, CA). A p value < 0.05 was taken to indicate statistical significance. The data correspond to the mean values ± SD.

**RESULTS**

**Moderate Exposure to Arsenic Increases the Formation of Atherosclerotic Lesions in ApoE−/− Mice**

In order to investigate the in vivo effect of arsenic on atherosclerosis formation, we exposed ApoE−/− mice to moderate arsenic concentrations. We used the ApoE−/− mouse model because these mice develop atherosclerotic plaques, in contrast to wild-type mice, providing a validated tool for atherosclerosis research. ApoE−/− mice were exposed to 200 or 1000 ppb arsenic for either 8 or 13 weeks and atherosclerotic lesion formation was examined en face in the aortic arch and in cross-sections of the aortic sinus (Fig. 1). Interestingly, mice fed a normal diet for 13 weeks and given 200 ppb arsenic had more plaque formation in the aortic arch compared with both controls and 1000 ppb arsenic-exposed groups (Figs. 1A and 1B). In the aortic sinus, the increase in plaque formation was already seen after 8 weeks of 200 ppb arsenic exposure (Figs. 1C and 1D) and was exacerbated after 13 weeks exposure. Again, like in the arch, lesions from 200 ppb arsenic-exposed mice were significantly greater than both those from control and 1000 ppb arsenic-exposed mice.

We also exposed mice to high-fat diet with and without arsenic in the drinking water. All high-fat–fed mice already had significant lesions after 8 weeks, and although the 200 ppb arsenic-exposed animals tended to have increased lesion size, this was not statistically significant, even with longer arsenic exposure (Supplementary Fig. 1).

**Arsenic Alters Plaque Composition**

In order to determine whether arsenic influences plaque composition, we assessed different constituents of the plaque. Smooth muscle cells proliferate in lesions and synthesize collagen, forming a more stable plaque. In contrast, high lipid content is associated with less stable plaque. We found that 200 ppb arsenic had the most dramatic effect on plaque composition. In mice fed either a normal or a high-fat diet, 200 ppb arsenic exposure was associated with lower smooth muscle cell content in their entire plaque area (Fig. 2, p < 0.01) and reduced collagen staining (Fig. 3, p < 0.05) as compared with control mice. This difference in plaque phenotype was observed at both 8 and 13 weeks. The higher 1000 ppb arsenic concentration significantly decreased smooth muscle cell content only in animals fed a normal chow (Fig. 2A, p < 0.05) and did not influence collagen content in any animals, regardless of time point or of diet (Fig. 3). Macrophage composition of the plaque also was evaluated and no significant quantitative changes in the percentage of macrophage staining relative to the whole plaque area were seen between controls and arsenic-exposed animals (Supplementary Fig. 2).

Furthermore, we determined the lipid content within the lesions by staining the aortic sinus with oil red O. Significantly, in mice fed a normal diet, both 200 and 1000 ppb arsenic increased plaque lipid content at both 8 and 13 weeks (Fig. 4). Notably, 1000 ppb arsenic increased lipid content without increasing plaque size (Fig. 1). When fed a high-fat diet, mice exposed to arsenic had increased lipid content in the lesions, but this change was lost over time (Fig. 4). Although the lipid content within the plaque is altered by arsenic exposure, the circulating levels (Table 1) as well as the total cholesterol/HDL ratio (data not shown) were not significantly altered. As previously reported, high-fat diet did not alter triglycerides levels (Joven et al., 2007) (Table 1). However, in mice fed a normal diet, exposure for 8 weeks to 200 ppb arsenic caused a significant increase of the plasma triglyceride levels (Table 1), which is a risk factor for atherosclerosis (Carmena et al., 2004). In contrast to the changes observed in triglycerides, arsenic did not alter either the AST and ALT liver enzyme activities (data not shown) or animal weight (Table 1), consistent with a previous report with higher arsenic exposure in C57Bl/6 mice (Arteel et al., 2008).

**Genes That Regulate Lipid Homeostasis are Inhibited In Vivo by Arsenic**

We previously showed that arsenic inhibits LXRs, and this correlated with decreased cholesterol efflux in vitro (Padovani et al., 2010). Thus, we hypothesized that the increased lipid content that we observed in the 200 and 1000 ppb arsenic-exposed groups at 8 weeks would correlate with decreased expression of LXR target genes in macrophages. We focused our analyses on LXR target genes that control adequate cholesterol clearance in the macrophages: (1) the ATP-binding cassette A1 (ABCA1) responsible for the efflux of cellular cholesterol and phospholipids (Banowski, 2008), (2) the phospholipid transfer protein (PLTP), which transfers triglyceride-rich lipoproteins to HDL (Mak et al., 2002), (3) the adipocyte fatty acid–binding protein (aP2) (Liu et al., 2007) and (4) the sterol response element–binding protein 1 (SREBP-1c) (Banowski, 2008). Mice were exposed to arsenic (200 and 1000 ppb) for 8 weeks, and expression of LXR target genes was assessed in thioglycollate-elicited macrophages. In mice fed a normal diet, 200 ppb arsenic significantly (p < 0.001) reduced macrophage messenger RNA (mRNA) expression of ABCA1, PLTP, and aP2 (Figs. 5A–C). ABCA1 and aP2 were also downregulated in mice exposed to 1000 ppb arsenic (Figs. 5A and 5C, p < 0.01), whereas SREBP-1c expression was not modified by arsenic exposure (Fig. 5D). In
FIG. 1. Five-week-old ApoE−/− mice were either exposed to two different concentrations of arsenic (200 or 1000 ppb) for 8 or 13 weeks or maintained on tap water. Mice received also normal chow. The aorta was cut with the luminal surface facing up, and the inner aortic surface was stained with oil red O (A and B). Percent of lesion area of the aortic arch (from the heart to the first intercostal artery) was evaluated with the Infinity Analyze software 5.0 (Lumenera). In C and D, sections of the aortic sinus were stained with oil red O, and percentage of the lesion area was evaluated relative to the total aortic sinus area. Representative pictures from the 13 weeks exposure experiments are shown. Values are expressed as mean ± SD. *p < 0.05; **p < 0.01 relative to their own control.
animals fed a high-fat diet, the basal mRNA levels of ABCA1, PLTP, aP2, and SREBP-1c were all enhanced, as expected (Baranowski, 2008). However, arsenic exposure did not alter mRNA expression of these LXR target genes in the fat-fed mice (data not shown).

**Arsenic Increases the Circulating Levels of Proinflammatory Cytokines MIP1α and IFNγ**

Exposure to arsenic increases several proatherogenic inflammatory cytokines (Bunderson et al., 2004; Srivastava et al., 2009; Straub et al., 2009). Thus, we assessed the levels of
the MIP1α and IFNγ, both of which are known to enhance atherosclerosis (Ardigo et al., 2007; McLaren and Ramji, 2009). MIP1α and IFNγ concentrations were analyzed in the plasma of mice exposed to 200 and 1000 ppb arsenic for 8 weeks and fed a normal diet. Interestingly, protein was increased in the plasma of mice exposed to 200 ppb arsenic (Fig. 6A). This correlated with an increase in MIP1α mRNA expression in thioglycollate-elicited macrophages from mice fed a normal diet exposed to 200 ppb arsenic (Supplementary Fig. 3). Plasma IFNγ was also increased in the 200 ppb exposed group (Fig. 6B). These results indicate that arsenic modulates proinflammatory cytokine production at a time that coincides with the early stage of atherosclerosis formation.

**DISCUSSION**

Numerous epidemiological data link arsenic exposure to an increased risk of atherosclerosis and cardiovascular mortality.
Similarly, experimental data in mouse models suggest that arsenic accelerates atherosclerosis (Simeonova et al., 2003) and that even short-term early-life exposure to arsenic, both in utero and postnatally, can increase lesion formation (Srivastava et al., 2007, 2009). Despite the clear epidemiologic links between arsenic and an increased risk of atherosclerosis, the mechanisms by which arsenic enhances atherosclerosis are unclear. Based on our in vitro data (Padovani et al., 2010) and epidemiologic reports (Engel and Smith, 1994; Medrano et al., 2010), we hypothesized that moderate arsenic concentrations would be proatherogenic in vivo. We observed an increase in lesion formation in the aortic valves and in the aortic arch following arsenic exposure in the ApoE\(^{-/-}\) mice fed a normal diet. In the aortic sinus, the increase in plaque formation

**FIG. 4.** Five-week-old ApoE\(^{-/-}\) mice were either exposed to two different concentrations of arsenic (200 or 1000 ppb), for 8 or 13 weeks or maintained on tap water. Mice received also either normal chow (A and C) or high-fat diet (B and D). Sections of the aortic sinus were stained with oil red O. The lipid content was evaluated relative to the total lesion area. Representative pictures from the 13 weeks exposure experiments are shown. Values are expressed as mean ± SD. *\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\) relative to their own control.
was already significant after 8 weeks of 200 ppb arsenic exposure and the increase was even more evident after 13 weeks exposure, at which time, effects on the aortic arch were also significant. We also tested whether arsenic-enhanced atherosclerosis formation in mice fed a high-fat diet. Indeed, high fat–fed animals developed more atherosclerosis than mice fed a normal chow.

### TABLE 1

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<th>Arsenic (ppb)</th>
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<td>Triglyceride levels (mg/dl)</td>
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<td>138 ± 18</td>
<td>28.6 ± 0.9</td>
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<td>125 ± 6</td>
<td>30.2 ± 1.4</td>
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<td>200</td>
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<td>233 ± 29**</td>
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**Note.** ND, Not determined.

FIG. 5. Five-week-old ApoE<sup>−/−</sup> mice were either exposed to two different concentrations of arsenic (200 or 1000 ppb), for 8 weeks or maintained on tap water and fed with normal diet. Total RNA from elicited macrophages was isolated and mRNA gene expression was assessed by real-time reverse transcription–PCR of the following LXR target gene: ABCA1 (A), PLTP (B), aP2 (C) and SREBP-1c (D). Values are expressed as mean ± SD. **p < 0.01; ***p < 0.001; relative to their own control. Each sample (n = 4) was analyzed in triplicate (technical replicate) and expressed relative to the 36B4 housekeeping gene. a.u. arbitrary units.
as expected. Moreover, we observed a comparable 10-fold increase in percent lesion area after 13 weeks with a 15% fat (1.25% cholesterol) diet to the 11-fold increase reported previously with a 20% fat (1.0% cholesterol) diet for 14 weeks (Joven et al., 2007). Although there was a trend toward increased lesion size, arsenic did not significantly alter high-fat diet–induced lesion area. This is not surprising considering previous data showing that high arsenic exposure (20–100 ppm) accelerates plaque formation under regular chow; however, this effect is lost after the addition of more than 4 weeks of an atherogenic diet (Simeonova et al., 2003). We also observed a significant increase in plasma triglycerides when mice fed a normal diet were exposed to 200 ppb, but not 1000 ppb, arsenic for 8 weeks. This finding is surprising considering data showing that circulating triglycerides decreased in ApoE−/− mice exposed to 1000 ppb arsenic for 13 weeks (Srivastava et al., 2009), although this could be attributed to the difference in timing.

Furthermore, we evaluated the effect of arsenic on plaque composition in our mouse model. Exposure to arsenic decreased the quantity of smooth muscle cells and collagen early in the plaque development, a phenotype which persisted at 13 weeks. The 200 ppb arsenic-exposed mice exhibited significantly decreased smooth muscle cell and collagen contents within the plaque, whereas the lipid content within the lesions increased with arsenic exposure. Importantly, these changes were seen even in the context of a high-fat diet, even though arsenic altered neither the circulating lipid level nor the size of the lesions. The observed modifications in plaque composition suggest that arsenic changes the atherosclerotic lesion to an advanced phenotype (Stary et al., 1995), by making them less stable (more lipids, less collagen, and less smooth muscle cells) and potentially more dangerous (Shah, 2003). Intriguingly, arsenic exposure, even to relatively low levels (less than 100 ppb), has been linked epidemiologically in humans to cerebrovascular disease, such as stroke (Lisabeth et al., 2010; Medrano et al., 2010). These data suggest that arsenic exposure is a risk factor for cardiovascular disease independent of lipid profile.

The arsenic-enhanced lesion is correlated with changes in several proatherogenic parameters. We showed a significant rise in the plasma levels of the proinflammatory proteins MIP1α and IFNγ in the 200 ppb arsenic-exposed group. IFNγ is known to potentiate atherosclerosis formation in both mouse models and in vitro (McLaren and Ramji, 2009), whereas MIP1α is involved in inflammation and correlates with increased atherosclerosis (Ardigo et al., 2007). Although arsenic-enhanced lesion formation did not correlate with changes in total macrophage content with the plaque, we do show that arsenic exposure in vivo may result in altered macrophage function, as indicated by the decreased LXR target gene expression. Our previous in vitro results show that arsenic inhibits LXR activity in macrophages resulting in a decreased LXR target gene expression and a decreased cholesterol efflux (Padovani et al., 2010). Thus, even in the absence of total macrophage content changes, the decreased LXR target gene expression could have the potential to enhance lesion formation by decreasing cholesterol efflux from those cells. In addition, it is possible that arsenic alters macrophage phenotype within the plaque without altering the number of resident macrophages. Different macrophage phenotypes (M1 and M2) are present within atherosclerotic lesions, and their ratios may predict the evolution of the lesion.
of M1 macrophages (Mantovani et al., 2009). Interesting, MIP1α may be a marker of M1 macrophages (Mantovani et al., 2004), suggesting that we may find an increase in M1 macrophages within the plaque in our future studies.

Previous reports showed a dose-dependent increase in plaque formation when mice fed a normal diet were exposed to arsenic at 1000 ppb and over (Srivastava et al., 2009). Although we find more atherosclerotic plaque with a five times lower concentration, our results with 1000 ppb arsenic concur with the extent of lesion formation in the aortic arch observed with a 13 weeks arsenic exposure on ApoE−/− mice (1- to 5-fold increase) (Srivastava et al., 2009). Surprisingly, the increase in atherosclerotic lesion area in the aortic sinus with the lower 200 ppb arsenic exposure resulted in a 3-fold increase, which was equivalent to a 2- to 4-fold increase reported with a 49 ppm concentration (Srivastava et al., 2009). In agreement with the extent of lesion formation, the lower arsenic concentration inhibited LXR target gene expression more profoundly than the higher exposure. Moreover, we found that exposure to 200 ppb arsenic increases circulating plasma triglycerides and induces a proinflammatory cytokine release, whereas the higher 1000 ppb did not. Interestingly, this is not the first example of nonmonotonic responses to arsenic. Arsenic-induced changes in peripheral lymphocyte proliferation follow a bell-shaped curve, with the highest changes found at 75 ppb (Meng, 1993). Furthermore, arsenic decreased extracellular matrix proteins in fibroblast more profoundly at lower doses (10 and 25 ppb) than higher concentrations (Hays et al., 2008). We hypothesize that different mechanisms of action may be involved in moderate and high exposure arsenic-enhanced atherosclerosis in mice. Higher exposures may be more cytotoxic, inducing plaque progression through macrophage apoptosis or necrosis (Clarke et al., 2010). Recently, differential vascular smooth muscle cell cytokine production has been linked to either apoptosis or necrosis within atherosclerotic lesions (Clarke et al., 2010). Further investigation is required to link dose-dependent lesion formation to cytokine profile and mechanisms of cell death.

Our data suggest that a moderate environmental arsenic concentration of 200 ppb, to which many people are exposed worldwide (Brunt et al., 2004; Nordstrom, 2002), can cause deleterious cardiovascular effects by enhancing atherosclerotic plaque formation characterized by less stable lesions. Moreover, because atherosclerosis formation was significant in arsenic-exposed mice fed a normal diet, it suggests that arsenic may have proatherogenic effects in populations that are not traditionally considered at risk based on serum lipid profile. All together, these data suggest that environmental concentrations of arsenic may be a greater health risk than previously anticipated.

SUPPLEMENTARY MATERIAL

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

FUNDING

National Institute of Health (NIH #R21ES014911-02 to K.K.M.); Canadian Institutes of Health Research (CIHR MOP110931 to K.K.M.; CIHR #210176 to S.L.; MOP82790 to E.L.S.). M.L. is supported by the Canadian Institutes of Health Research/Fonds de la recherche en santé du Québec training grant FRN53888 of the McGill Integrated Cancer Research Training Program.

ACKNOWLEDGMENTS

The technical assistance of André Turgeon for the cytokine analyses is thankfully acknowledged. Disclosure: The authors declare that there are no conflicts of interest.

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