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EFFECTS OF ENVIRONMENTAL LEAD CONTAMINATION ON CATTLE IN A LEAD/ZINC MINING AREA: CHANGES IN CATTLE IMMUNE SYSTEMS ON EXPOSURE TO LEAD IN VIVO AND IN VITRO

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Abstract—The Republic of Zambia is rich in mineral resources, such as zinc (Zn) and lead (Pb), and mining is a key industry in Zambia. A previous study of Pb pollution in Kabwe, one of the main mining areas, found that soil was contaminated with high levels of toxic metals over a substantial area. In the present study, the authors focus on toxic metal pollution in cattle, one of the most important domestic animals in Zambia. Blood samples from cattle in Kabwe and a control area (Lusaka) were tested for toxic metal content. They also measured mRNA expression of metal-responsive proteins and cytokines in white blood cells using real-time reverse transcriptase polymerase chain reaction. In the present in vitro study, The authors cultured peripheral blood mononuclear cells (PBMCs) from cattle, exposing them to Pb acetate for 24 h and analyzing mRNA expression of metal-responsive proteins and selected cytokines. Lead concentrations in cattle blood from Kabwe were significantly greater than those from Lusaka, as were the mRNA expressions of metallothionein-2 (MT-2), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-1β (IL-1β), IL-6, and inducible nitric oxide synthase (iNOS). The present in vitro study demonstrated that Pb exposure led to an increase in the expressions of MT-2, TNF-α, IL-1β, and iNOS, similar to those found in vivo. These results indicate the possibility of immune system modulations in cattle from the Kabwe area. Environ. Toxicol. Chem. © 2012 SETAC

Keywords—Cattle Cytokines Immune system Lead Metallothionein

INTRODUCTION

Many African countries have abundant underground resources including industrially important rare metals, which are among the main economic resources for these countries [1]. Following recent rapid economic development in these areas, chemical pollutants such as metal contaminants are now a major health hazard [2,3].

The economy of the Republic of Zambia depends on a limited number of commodities, with copper accounting for 60% of exports. The country holds vast mineral resources, including copper (Cu), cobalt (Co), zinc (Zn), and lead (Pb), and has seen significant growth in the mining industry, with the majority of these metals also smelted within the country [4]. The Kabwe mine, one of the major mining areas in Zambia, was operational for over 90 years (until June 1994) and was the main producer of Pb and Zn in Zambia [5]. It produced a total of 1.8 million tons of Zn, 0.8 million tons of Pb, and relatively small amounts of other metals such as Cd (235 tons), silver (79 tons), Cu (64 tons), and fused vanadium oxide (7,820 tons) [6,7]. Accordingly, metal pollution has become a serious environmental issue in Zambia, with health implications for humans and wildlife [3,5,8]. Exposure to toxic metals such as Pb, Cd, and arsenic (As) can cause serious health problems in both groups [9–11]. It is therefore important to estimate the toxicological effects and impact of environmental metal contamination.

In an earlier survey of Pb pollution in Kabwe, soil was found to be contaminated with high levels of toxic metals over a substantial area [5,12]. Lead levels over 65 μg/dl, which can induce subacute toxicological effects, were found in the surrounding communities in 650 cases [5]. A report by the Blacksmith Institute ranked Kabwe among the 10 most polluted places in the world [3]. Despite these alarming reports of metal pollution in Kabwe, the relationship between metal contamination in soil and animals has not yet been thoroughly investigated [2,8]. Numerous authors report that toxic metals such as Pb affect the immune system, even in low concentrations [13]. In fact, immune suppression due to toxic metal exposure was reported as an important factor in viral carcinogenesis in aquatic wildlife [14]. It was also reported that the toxicity of certain metals caused alterations in cytokines; high concentrations of Pb exposure caused immunosuppression, increased immunoglobulin G (IgG) production, and abnormal activation of inflammatory cytokines [15]. Faith et al. [16] and Bunn et al. [17] reported that Pb exposure caused the suppression of cell-mediated immune processes. In our previous study, soil highly contaminated with Pb was found in the Kabwe area [18]. Wild

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rats and domestic cattle also contained high concentrations of Pb in their livers and kidneys, compared with those of uncontaminated areas [18,19]. The high level of metal contamination strongly suggests the possibility of toxicological effects on wildlife and domestic animals in this area.

In the present study, we investigated the accumulated levels of toxic metals and their effects on metal-responsive proteins and cytokines in cattle, one of the most widespread and important domestic animals in Zambia.

MATERIALS AND METHODS

Study sites and sampling

The present study was conducted in two areas, Kabwe and Lusaka. Kabwe is 130 km north of Lusaka, the capital city of Zambia. A Pb and Zn mine in Kabwe was in operation until 1994. Peripheral blood (30–50 ml) was collected from 12 female cows (crossbred) living around the mine in Kabwe (adults, ~36 months old; they lived at least 30 months in the area), and from 10 female cows (crossbred) raised on a farm at the University of Zambia (UNZA), Lusaka (adults, ~36 months old). Both groups grazed on their respective local pastures, and were fed commercial feed containing maize from various parts of the country and supplements manufactured in Zambia. Samples from Lusaka were used as controls, as UNZA is remote from likely sources of pollution such as freeways, industrial facilities, and mining areas (concentrations of Pb, DDT, and benzo[*a*]pyrene in the soil were less than 20 ppm, 10 ppb, and 50 ppb, respectively), and the health of the cattle was well maintained by faculty staff. Animals were under the care of district veterinary officer (DVO) and were healthy and without clinical symptoms of infectious diseases.

Preparation of peripheral white blood cells

A quantity of peripheral blood of 0.5 ml was mixed thoroughly with 0.8 ml of cell lysis solution (NH_4Cl 8.26 g/L, $NaHCO_3$ 1.19 g/L, ethylenediamine tetraacetic acid-2Na 0.0378 g/L). After 10 min at room temperature, the samples were centrifuged at 5,000 *g* for 5 min and the supernatant was discarded. To wash the pelleted peripheral white blood cells, 0.8 ml of cell lysis solution was added and the centrifugation was repeated. After discarding the supernatant, 0.7 ml of RNA*later* (Sigma-Aldrich) was added, and samples were stored at −20°C until RNA extraction was performed.

Extraction and measurement of metals

Metals were extracted from the blood by acid digestion, using a method modified from that used by Nakayama et al. [18]. Briefly, 10 to 30 g of fresh blood was placed in a 200-ml flask. Nitric acid (20 ml) and perchloric acid (5 ml) were then added. The samples were gradually heated to 200°C on a hotplate for up to 24 h and then left for 6 h to evaporate to approximately 1 ml. Next, 0.2 ml of lanthanum chloride (100 g/L) was added, and the volume was made up to 20 ml with 2% HNO_3. A reagent blank was produced using the same procedure.

After preparation of the calibration standard, the concentrations of six elements (chromium [Cr], Co, Cu, Zn, Pb, and Ni) in the blood samples were measured using an atomic absorption spectrophotometer (AAS; Z-2010, Hitachi High-Technologies) with an acetylene flame or argon nonflame. Cadmium was measured by inductively coupled plasma-mass spectrometry (ICP-MS; HP4500, Agilent). The overall recovery rates (mean ± SD) of Cr, Co, Cu, Zn, Cd, Pb, and Ni were 91 ± 3.0, 92 ± 3.4, 89 ± 5.6, 91 ± 2.3, 111 ± 8.3, 90 ± 3.5, and 92 ± 4.2%, respectively. The detection limits ($\mu g\, kg^{-1}$) of Cr, Co, Cu, Zn, Cd, Pb, and Ni were 0.5, 0.5, 1.0, 0.1, 0.2, 1.0, and 0.5, respectively. Each metal concentration was converted from $mg\, kg^{-1}$ wet weight to $mg\, kg^{-1}$ dry weight by subtracting the mass of the water content.

Measurement of mRNA expression

Levels of metallothionein-2 (MT-2), heme oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS), and cytokines (interferon-γ [IFN-γ], interleukin-6 [IL-6], IL-1β, and tumor necrosis factor-α [TNF-α]) mRNA expression were measured using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was extracted from peripheral white blood cell samples stored in RNA*later* (Sigma-Aldrich), using TRI reagent (Sigma-Aldrich). First-strand cDNA was synthesized from total RNA with ReveTra Ace (Toyobo) and oligo-dT primers (Toyobo).

The primers used for measurement of MT-2, HO-1, iNOS, and cytokine expression levels are shown in Table 1. β-Actin was used as a reference gene. The measurement was conducted on a StepOnePlus real-time polymerase chainr (PCR) system (Applied Biosystems) using Thunderbird SYBR qPCR mix (Toyobo). Each sample was analyzed in duplicate. The following cycling parameters were used: an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and then at 57 to 63°C for 1 min (Table 1). Melting curves were performed to verify that only a single product with no primer–dimers was amplified. Relative quantity of gene expression was calculated from real-time PCR data (cycle threshold value) by the comparative cycle threshold method. Amplification efficiency was within 95 to 110% for each gene. The sequences of the PCR products were confirmed using an ABI Prism 310 genetic analyzer (Applied Biosystems).

In vitro exposure of peripheral blood mononuclear cells (PBMCs) to lead

Peripheral blood samples were obtained from the neck vein of cattle kept in the Faculty of Veterinary Medical Science, Hokkaido University, Japan. Cattle were Holstein females approximately 30 months old, healthy, and without the clinical symptoms of infectious diseases. They were fed with commercial feed supplements. Peripheral blood mononuclear cells were purified from heparinized venous blood by density gradient centrifugation (1,600 *g* for 20 min) using 60% Percoll (540 ml Percoll; GE HealthCare), mixed with 200 ml of 5 ml Hank's balanced salt solution (HBSS; Sigma) and 260 ml of MilliQ water. The next step was the addition of 1 ml of cell lysis solution (NH_4Cl 8.26 g/L, $NaHCO_3$ 1.19 g/L, ethylenediamine tetraacetic acid-2Na 0.0378 g/L) to the PBMC fraction to hemolyze the red blood cells. After washing the PBMC with 30 ml of phosphate-buffered saline, the suspension was centrifuged (400 *g* for 10 min). After the supernatant was discarded, Roswell Park Memorial Institute 1640 medium (Sigma) was added to the pelleted PBMC fraction. Once suspended in the medium, cells were counted by an erythrocytometer. Cell counts were adjusted to 5×10^6 cells/ml for each well of a 24-well plate.

The PBMCs were preliminarily incubated at 37°C for 1 h in complete Roswell Park Memorial Institute 1640 medium (Sigma), with a 1% antibiotics mix (final concentrations for each antibiotic: 1,000 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B; Nacalai Tesque) and 10% fetal bovine serum (Gibco), in a humidified atmosphere of 95%

Table 1. Primers, annealing temperature, and size of amplified fragment for metal-responsive proteins and selected cytokines

	Primers/sequences $5' \rightarrow 3'$	Annealing temperature (°C)	Size of amplified fragment (bps)	Reference
MT-2				
	Forward/GCAGCTGCTGTGCCTGAAG Reverse/AAAATATGCAGGTTTGTACACGTTGT	63	77	[34]
IFN-γ				
	Forward/AGCCAAATTGTCTCCTTCTACTTC Reverse/CTGACTTCTCTTCCGCTTTCTG	63	261	[35]
β-Actin				
	Forward/CGCACCACTGGCATTGTCAT Reverse/TCCAAGGCGACGTAGCAGAG	60	227	[36]
IL-6				
	Forward/AGCGCATGGTCGACAAAATCT Reverse/GCCCAGTGGACAGGTTTCTG	63	523	[35]
IL-1β				
	Forward/ACCTTCATTGCCCAGGTTTCT Reverse/CTGTTTAGGGTCATCAGCCTCAA	57	120	[37]
TNF-α				
	Forward/TAACAAGCCGGTAGCCCACG Reverse/GCAAGGGCTCTTGATGGCAGA	60	277	[38]
iNOS				
	Forward/ACCTACCAGCTGACGGGAGAT Reverse/TGGCAGGGTCCCCTCTGATG	63	316	[39]
HO-1				
	Forward/GTGAGCTGACCCAAGAAGGTTT Reverse/CCTCCAGGGCCACATAGATG	63	69	[40]

MT-2 = metallothionein-2; IFN-γ = interferon-γ; IL = interleukin; TNF-α = tumor necrosis factor-α; iNOS = inducible nitric oxide synthase; HO-1 = heme oxygenase-1.

air plus 5% CO_2. After preliminary incubation, we changed the medium to a lead acetate–containing medium (0, 3, 6, 12, 25, or 50 μM lead acetate), and exposed the PBMC fraction to Pb for 24 h. No adjuvant was used to stimulate the PBMCs. Cell viability was measured using Cell Counting Kit-8 (Dojindo). Additionally, trypan blue staining was used to distinguish between viable and dead cells.

After incubation, total RNA was isolated from cells using Tri Reagent (Sigma) according to the manufacturer's protocol. The expression levels of MT-2, HO-1, iNOS, and selected cytokines were measured as described above.

Statistical analysis

Statistical analysis was performed using the JMP 7.0.1 package (SAS Institute). Data were normalized by log10 transformation. Differences between the Kabwe and Lusaka cattle groups were analyzed using a Student's *t* test. Pearson product-moment correlation was used to analyze the relationship between metal concentrations in the blood and mRNA expression levels. For assessment of differences among the in vitro exposure groups, Dunnett's test was used. Tests were evaluated at a statistical significance level of $p < 0.05$.

RESULTS

Metal concentrations in cattle peripheral blood

No differences were found in accumulated Cr, Cu, Ni, or Zn concentrations between cattle from Lusaka and Kabwe ($p > 0.05$; Fig. 1). In contrast, greater concentrations of Pb (9.0 ± 4.7 and 90.6 ± 67.6 μg/kg dry wt, respectively) and Cd (15.7 ± 15.7 and 114.9 ± 74.6 ng/kg dry wt, respectively) were observed ($p < 0.001$ for both Pb and Cd). However, no Co was detected in cattle blood in the present study.

Relationships among the metal concentrations were analyzed (Table 2). Positive correlations between Zn and Ni or Cu ($r = 0.43$ and 0.52, respectively) were detected. In addition, positive correlations between Cu and Cd or Pb ($r = 0.45$ and 0.43, respectively) were found. A strong correlation was found between the Pb and Cd accumulation levels in cattle blood ($r = 0.80$, $p < 0.001$).

mRNA expression of MT-2, HO-1, iNOS, and cytokines in cattle white blood cells

The expression levels of MT-2 in cattle white blood cells from the Kabwe area were greater than those in the white blood cells of cattle from Lusaka ($p < 0.05$; Fig. 2). However, there was no difference in the expression levels of HO-1 in the cattle from the two areas ($p < 0.05$). The expression levels of iNOS

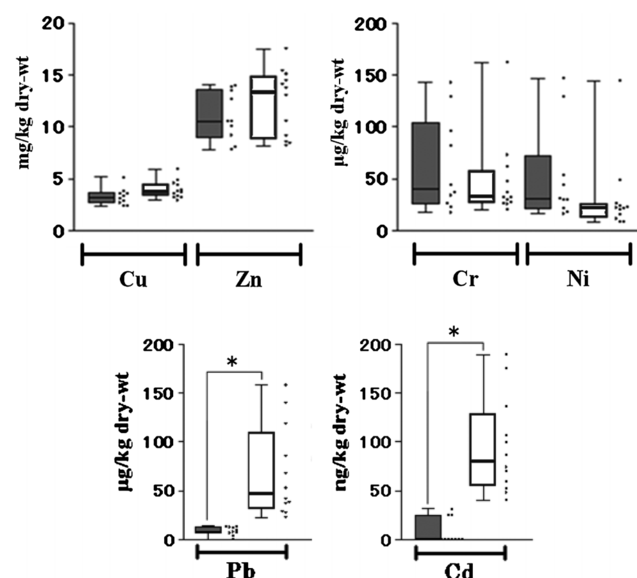

Fig. 1. Metal concentrations in blood from cattle in Kabwe and Lusaka. Closed bars show data from Kabwe and open bars data from Lusaka. Asterisk indicates a significant difference between Kabwe and Lusaka (Student's *t* test, $p < 0.05$). Concentrations are shown as mg/kg dry weight (Cu and Zn), μg/kg dry weight (Cr, Ni, and Pb), and ng/kg dry weight (Cd).

Table 2. Correlation coefficients (*r*) among metal concentrations, MT-2, iNOS, HO-1, and cytokine mRNA expression levels

	Cu	Zn	Cr	Ni	Pb	Cd	MT-2	IL-6	IL-1β	TNF-α	IFN-γ	iNOS	HO-1
Cu	1												
Zn	0.52*	1											
Cr	0.10	0.01	1										
Ni	0.04	0.43*	0.11	1									
Pb	0.43*	0.38	−0.03	−0.12	1								
Cd	0.45*	0.27	−0.12	−0.06	0.80*	1							
MT-2	0.16	0.30	0.12	−0.18	0.48*	0.34	1						
IL-6	0.23	0.15	0.27	−0.34	0.47*	0.32	0.59*	1					
IL-1β	0.22	0.1	0.34	−0.23	0.39	0.22	0.61*	0.91*	1				
TNF-α	0.18	0	0.34	−0.43*	0.55*	0.45*	0.53*	0.76*	0.77	1			
IFN-γ	−0.33	−0.05	0.14	0.14	−0.36	0.39	0.02	−0.34	−0.16	−0.11	1		
iNOS	0.46*	0.19	−0.03	−0.4	0.62*	0.53*	0.64*	0.78*	0.72*	0.61*	−0.41	1	
HO-1	−0.18	−0.18	−0.29	−0.49*	−0.28	−27	−0.19	−0.17	−0.13	−0.27	0.25	−0.19	1

*Values significant at the $p < 0.05$ level.
MT-2 = metallothionein-2; IL = interleukin; TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ; iNOS = inducible nitric oxide synthase; HO-1 = heme oxygenase-1.

were markedly greater in the blood of Kabwe cattle than in that of cattle from Lusaka (2–1,300-fold). In the case of cytokines, the expression levels of IL-1β, IL-6, and TNF-α in particular were greater in Kabwe cattle compared with cattle from Lusaka. The expression levels of IFN-γ mRNA were lower in the white blood cells of cattle from the Kabwe region.

Correlations among metal concentrations and mRNA expression levels were also analyzed (Table 2). Positive correlations between Pb concentration and expression levels of MT-2, IL-6, TNF-α, and iNOS were detected ($r = 0.48$, 0.47, 0.55, and 0.62, respectively). Correlations between levels of accumulated Cd and TNF-α and iNOS expression ($r = 0.45$ and 0.53, respectively) were also detected. In addition, Cu concentration in blood showed a positive relationship with iNOS expression ($r = 0.46$). In contrast, negative relationships between Ni levels and the expression of TNF-α and HO-1 ($r =$ -0.43 and -0.44) were found.

Regarding correlations in mRNA expression, positive correlations between MT-2 expression levels and IL-6, IL-1β, TNF-α, and iNOS ($r = 0.59$, 0.61, 0.53, and 0.64, respectively) were found. No correlations were observed between HO-1 and the expression of other mRNAs.

Alterations in mRNA expression of MT-2, HO-1, iNOS, and cytokines in PBMCs exposed to Pb in vitro

No difference was found in cell numbers between Pb-exposed PBMC groups and the control group after the 24-h cell culture period (the survival rate was almost 100% for the 24-h exposure used in the present study). After cells were exposed to 50 μM Pb, MT-2 mRNA expression increased 7.7-fold compared with control cells (treated with vehicle; Fig. 3). In the case of iNOS, the mRNA expression level was elevated in response to exposure to both 25 and 50 μM Pb ($p < 0.05$).

Cytokine mRNA expression levels in PBMCs were also analyzed to identify the effects of Pb treatment on the immune function of mononuclear cells. Interleukin-1β and TNF-α mRNA expression was induced by Pb treatment, and it was found that the induction of these genes occurred in a dose-dependent manner. However, IL-6 expression levels in PBMCs were not elevated by Pb exposure, nor were changes in IFN-γ mRNA expression observed in cells after Pb treatment. In our preliminary study, PBMCs were also exposed to Cd (0, 3, 6, 12, 25, and 50 nM). No changes in MT-2, HO-1, iNOS, or cytokines were observed in the 0 to 50-nM range, which was considered to be the accumulated concentration range in cattle blood from Kabwe animals (data not shown).

DISCUSSION

In the present study, the concentrations of Cr, Cu, Ni, and Zn were not significantly different in blood samples from cattle of the Lusaka (control area) and Kabwe regions. The metals that were detected at markedly greater levels in Kabwe cattle blood were Pb and Cd. In our previous study, we showed that the source of Pb pollution was historical mining activity at the now abandoned Kabwe Pb/Zn mine [18,20]. We also found that

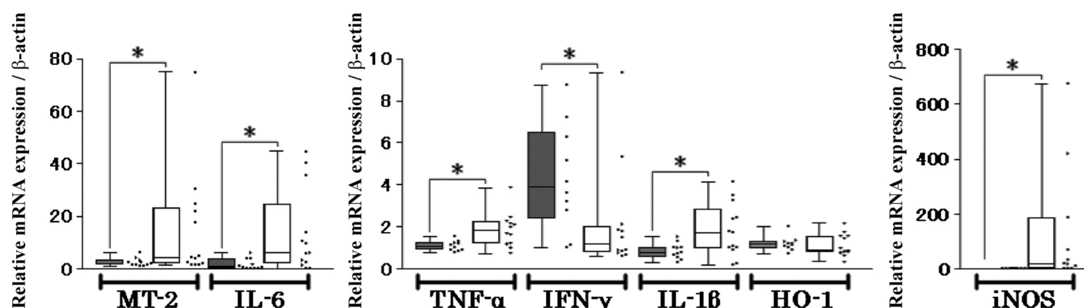

Fig. 2. mRNA expression levels of metallothionein-2 (MT-2), heme oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS), and the cytokines (interferon-γ [IFN-γ], interleukin-6 [IL-6], IL-1β, and tumor necrosis factor-α [TNF-α]) in cattle from Kabwe and Lusaka. Closed bars show data from Kabwe and open bars show data from Lusaka. Asterisk indicates a significant difference between Kabwe and Lusaka (Student's *t* test, $p < 0.05$).

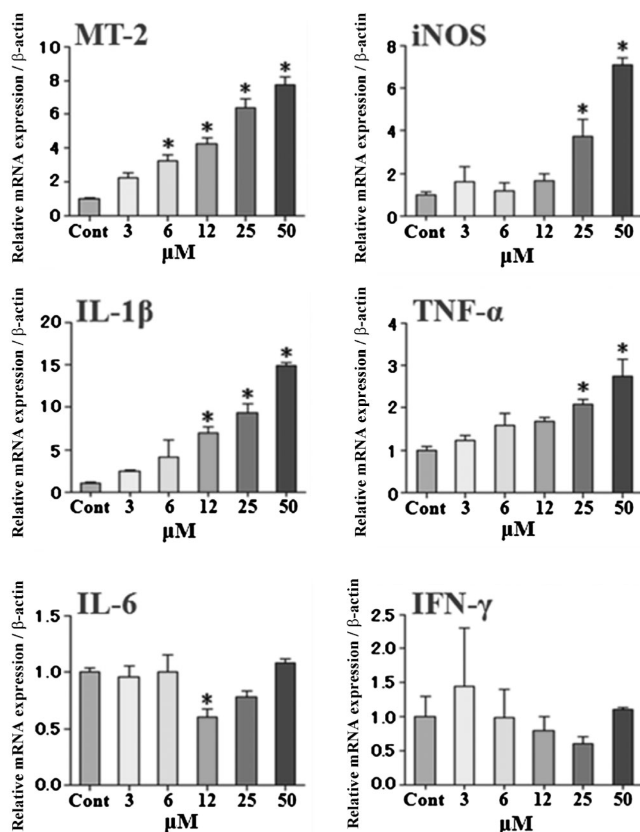

Fig. 3. mRNA expression levels of metallothionein-2 (MT-2), heme oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS), and the cytokines (interferon-γ [IFN-γ], interleukin-6 [IL-6], IL-1β, and tumor necrosis factor-α [TNF-α]) in peripheral blood mononuclear cells (PBMCs) exposed to lead. Asterisk indicates a significant difference compared with the control group (Dunnett's test).

concentrations of Pb and Cd in the livers and kidneys of cattle from Kabwe were greater than the benchmark values in offal destined for human consumption [19]. As blood samples had been collected from cattle that were kept in the northwest area, 10 km from the Kabwe mine, we suggested that contamination was due to Pb contamination in soil, air, and plants.

In the case of humans, the World Health Organization recommends that the levels of Pb in human blood should not exceed 100 μg/kg [21]. However, there have been few reports of Pb poisoning in cattle. Rice et al. [22] reported chronic Pb poisoning in cattle, demonstrating that aminolevulinic acid dehydratase activity was suppressed by exposure to Pb at a level of less than 100 μg/kg in blood. Thus it is possible that the sensitivity of cattle to Pb is similar to that of humans. In the present study, the average Pb concentration in cattle blood from the Kabwe area was 90.6 ± 67.6 μg/kg dry weight, suggesting that toxicological effects may be prominent in Kabwe cattle. A major toxic effect of Pb is developmental damage to the nervous system [23,24]. Recent studies have also shown toxicological effects of Pb on the immune system [11,25]. Several researchers have reported that tolerance to *Escherichia coli* and *Staphylococcus epidermidis* infection was reduced in laboratory animals after Pb exposure [26]. Lead exposure was also reported to enhance sensitivity to endotoxins [27]. Based on these reports, the high concentration of Pb in the most important domestic animal in the Kabwe area, the cow, indicates the importance of focusing on cattle immune system function in Pb-contaminated areas.

In our present research, we found the induction of mRNA expression of MT-2, iNOS, IL-6, TNF-α, and IL-1β, and the suppression of INF-γ in Kabwe cattle. Furthermore, we detected a positive correlation between Pb concentration and MT-2, iNOS, TNF-α, and IL-6 expression levels in cattle blood ($p < 0.05$). These results indicate that Pb exposure is one of the key agents modulating various transcription factors in cattle. In contrast, no difference in HO-1 mRNA expression levels was found between the two regions. It has been reported that HO-1 expression is regulated by NF-E2-related factor 2 via an antioxidant response element and is induced at the transcriptional level by heavy metal exposure [28], yet we found no correlation between Pb levels and HO-1 expression in the blood samples examined. Further research is necessary to shed light on this result.

It has been suggested that the immune toxicity of Pb is due to the abnormally increased production of TNF-α and IL-6 in macrophages exposed to it [29]. In addition, exposure of human PBMCs to Pb synergistically increased expression levels of TNF-α and its receptor TNF-R [30]. Increases in MHC class II expression levels may cause the abnormal alteration of cytokine production, especially an increase in inflammatory cytokines, in immune cells exposed to Pb [31]. The present study strongly suggests the possibility that the immune modulation in cattle sampled in the Kabwe area might be caused by Pb contamination. However, there are no reports of Pb toxicity in cultured cattle immunocytes. We therefore studied PBMC cultures to assess the possibility that Pb can cause immune disruption in cultured cattle blood cells. This finding is also supported by the fact that no genetic alterations resulting from PBMC exposure to Cd were found. Interestingly, the expression levels of inflammatory cytokines such as TNF-α and IL-1β were elevated after exposure to Pb in a dose-dependent manner.

In the present study, we also detected markedly high expression levels of iNOS in Kabwe cattle. Inducible nitric oxide synthase is induced by IL-1 and TNF in vivo and in vitro [32]. The iNOS expression levels seem to be coincident with alterations in the expression of inflammatory cytokines such as IL-1 and TNF.

IFN-γ is an important cytokine for antibody-mediated immunity against infectious diseases in the immune system [33]. In the present study, we found suppressed levels in Kabwe cattle contaminated with Pb. Our in vitro study, however, revealed that PBMC exposure to Pb did not cause a reduction in IFN-γ mRNA expression. A main reason for this can possibly be found in the differentiation cascade of T-helper 1 cells. The reduced levels of IFN-γ expression are caused by inhibition of the differentiation of T cells to T-helper 1 cells [31], and this differentiation is difficult to ascertain in PBMCs in vitro.

In conclusion, the present study has demonstrated the possibility that Pb contamination causes immunological alterations in cattle blood both in vivo and in vitro. In particular, the induction of MT-2, inflammatory cytokines, and iNOS mRNA was observed in cattle blood collected from the Pb-contaminated Kabwe mining region. We also showed that exposure of PBMCs to Pb caused the induction of MT-2 mRNA, inflammatory cytokines, and iNOS genes, supporting the results of the field study. We suggest the possibility that IFN-γ, which contributes to antibody-mediated immunity, is suppressed by Pb exposure in vivo. Our findings relating to the toxicological effects of Pb on the immune system of cattle also suggest the possibility that other animals, such as humans and wildlife species, may be subject to immune system disruptions caused by metal contamination around mining areas.

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