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Effect of non-toxic mercury, zinc or cadmium pretreatment on the capacity of human monocytes to undergo lipopolysaccharide-induced activation

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1 Metal salts can inhibit cell activity through direct toxicity to critical cellular molecules and structures. On the other hand, they can also change cell behaviour by inducing specific genes (including genes encoding members of the metallothionein [MT] gene family). Therefore, transition metals may affect cell functions either by acting as a toxin, or by transmitting or influencing signals controlling gene expression.

2 To explore the latter possibility, we measured the ability of low, non-toxic metal pretreatment to alter immune cell behaviour. We previously found that pretreatment of human monocytes with zinc induces metallothionein gene expression and alters their capacity to undergo a bacterial lipopolysaccharide-induced respiratory burst. We showed here that cadmium and mercury salts, at concentrations that exert no discernible toxicity, inhibit activation of human monocytic leukemia (THP-1) cells. $CdCl_2$ 1 μM, $ZnCl_2$ 20–40 μM or $HgCl_2$ 2 μM pretreatment for 20 h induced MT-2 mRNA and total MT protein accumulation and had no effect on proliferation potential or metabolic activity, but significantly inhibited the ability of subsequent lipopolysaccharide treatment to induce the oxidative burst, increased adhesion to plastic, and MT-2 and interleukin-1β (IL-1β) mRNA accumulation.

3 The phenomenon of metal-induced suppression of monocyte activation, at metal concentrations that have no effect on cell viability, has important implications for assessment of acceptable levels of human exposure to cadmium, zinc and mercury.

Keywords: Human monocyte; metal; mercury; cadmium; zinc; bacterial lipopolysaccharide; metallothionein

Introduction

Among toxic metals, cadmium and mercury have been the focus of intense interest. These metals are known to have deleterious cytotoxic effects in multiple mammalian organ systems (Pelletier *et al*., 1985; Friberg & Lener, 1986; Zalups & Lash, 1994). However, relatively little is known about the effect of low levels of these metals (that to not exert discernible toxicity) on immune cell function.

In contrast to their toxic effects, certain metals (including copper, iron, selenium, and zinc [Zn^{2+}]) are required for normal leukocyte development and function, including growth, differentiation and activation. Zinc, for example, can regulate interleukin-1 (IL-1) responsiveness in mouse thymocytes (Winchurch, 1988). Immune cell function is compromised in individuals suffering from zinc deficiency secondary to certain diseases (Moynahan, 1981; Castillo-Duran *et al*., 1987; Fabris *et al*., 1988). Zinc-deficient animals and man have reduced levels of markers of macrophage activation, including increased phagocytic capacity (Wirth *et al*., 1989), chemotaxis (Vruwink *et al*., 1991) and chemokinesis (Briggs *et al*., 1982). However, apparently contradictory effects of zinc supplementation have been described. For example, superoxide anion production following activation of guinea-pig peritoneal macrophages was depressed following *in vitro* zinc supplementation, but increased when animals were treated with zinc *in vivo* (Nakamura *et al*., 1987). Both stimulation (Briggs *et al*., 1982) and depression (Chvapil *et al*., 1977) of phagocytosis have been obtained following zinc treatment. And, while zinc supplementation enhanced humoral and cellular immunity in man (Hertel & Wibbertmann, 1990; Keen & Gershwin, 1990), it has been shown to inhibit leukocyte mobility (Kiremidjian-Schumacher *et al*., 1981), release of histamine from rat, peritoneal mast cells and blood basophils (Kazimierczak & Maslinski, 1974; Marone *et al*., 1981), and lipopolysaccharide (LPS)-induced monocyte activation *in vitro* (Leibbrandt & Koropatnick, 1994).

On the other hand, inorganic cadmium (Cd^{2+}) and mercury (Hg^{2+}) have mainly detrimental effects, including direct *in vitro* cytotoxicity as well as inhibition of antibody production and cytokine production and proliferation, in isolated primary peripheral blood mononuclear cells (Lawrence & Slavik, 1992; Lawrence & McCabe, 1995).

The immunomodulatory activity of metal ions generally occurs at concentrations much lower than that required for overt cytotoxicity (Lawrence & McCabe, 1995). Both enhancement and depression of specific activities have been observed. Although Cd^{2+} and Hg^{2+} can be toxic to leukocytes, they can enhance leukocyte function under some circumstances. Low concentrations of Hg^{2+} (1 μM) have been shown to increase B cell IgM production (Lawrence & McCabe, 1995) and Cd^{2+} has been found to suppress (Chowdhury & Chandra, 1989; Cifone *et al*., 1989) or enhance (Koller & Roan, 1977) NK cell activity, and suppress (Thomas *et al*., 1985) or enhance (Koller & Roan, 1977) macrophage activity. Hg^{2+} may be an immunostimulant, since it can trigger genetically-restricted immune complex glomerulonephritis (ICG) in some rodent strains (Aten *et al*., 1988; Reuter *et al*., 1989; Pelletier *et al*., 1990; Kubicka-Murany *et al*., 1993; Hultman *et al*., 1993; Monestier *et al*., 1994). Hg^{2+} is suspected to have the same effect in man since low levels correlate with the appearance of ICG (Tubbs *et al*., 1982; Oliveira *et al*., 1987). On the other hand, Hg^{2+} has been shown to inhibit activation of T and B cells at levels as low as 5 ng ml^{-1} (Shenker *et al*., 1993), indicating an immunosuppressive effect.

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Transition metals can induce genes important in immune cell function. For example, metallothionein (MT) is the primary transition metal-binding protein in eukaryotic cells (Andrews, 1990). It has been implicated in immunoregulation of lymphocytes (Lynes *et al*., 1993), and is essential for LPS-induced monocyte activation (Leibbrandt *et al*., 1994). Other genes, including acute phase proteins important in immune responsiveness (i.e., α_1-acid glycoprotein and C-reactive protein, c-myc, c-jun, and c-fos) are also induced by transition metals (reviewed by Koropatnick & Leibbrandt, 1995). The critical role that these gene products play in monocyte activation suggest that induction with transition metals may alter responsiveness to LPS.

Therefore, immunomodulation, not only cytotoxicity, may be a significant consequence of exposure to metal salts. We tested the hypothesis that treating human monocytes with low, non-toxic doses of the non-essential metals Hg^{2+} and Cd^{2+}, and the essential metal Zn^{2+}, affects the ability of the cells to be activated by bacterial lipopolysaccharide, as measured by oxygen free radical production, and IL-1β mRNA and MT mRNA and protein accumulation.

Methods

Cell lines and culture conditions

THP-1 cells (a non-adherent human monocytic cell line from a male infant with acute monocytic leukemia; American Type Culture Collection, Rockville, MD) were maintained in RPMI 1640 (GIBCO-BRL, Oakville, Ontario, Canada) with 10% Fetaclone (Hyclone Laboratories, Logan, UT) and 1.25 mM L-glutamine in a 37°C incubator under 5% CO_2. Fetaclone contains approximately 4 μM zinc.

Treatment with metal salts and bacterial lipopolysaccharide (LPS)

Metal salts Zn^{2+}, cadmium or mercury pretreatment involved a 20 h exposure to added 20 or 40 μM $ZnCl_2$, 1 or 10 μM $CdCl_2$ or 2 μM $HgCl_2$, in medium plus Fetaclone. At the end of metal exposure, cells were centrifuged and resuspended in fresh medium without added metal salts. Aliquots of cells were frozen for isolation of total cellular RNA and analysis of MT-2 and IL-1β mRNA as described below.

LPS THP-1 cells were treated with 40 μg ml^{-1} LPS (Sigma, St. Louis, MO) in RPMI 1640 medium plus 10% Fetaclone and glutamine. Control cells (without LPS) and cells treated for 30 min with LPS (the time of peak induced H_2O_2 production (Pick & Keisari, 1980)) were assessed for H_2O_2 production as described below.

Metal salts plus LPS Aliquots of cells pretreated with metal salts for 20 h were immediately treated with 40 μg ml^{-1} LPS as described above, and assessed for activation 30 min later by the phenol red assay. Aliquots of cells were frozen for isolation of total cellular RNA and analysis of MT-2 and IL-1β mRNA as described below.

Measurement of toxicity

Cytotoxicity of $HgCl_2$ 0–75 μM, $CdCl_2$ 1–10 μM or $ZnCl_2$ 0–40 μM was assessed by a growth assay, and by the metabolic alamarBlue assay (BioSource International, Camarillo, CA).

Growth assay THP-1 cells (25,000) were suspended in 1 ml of complete medium plus various concentrations of metal salts in 3 separate wells of a 24 well tissue culture plate (Nunc, BRL) and counted every 24 h for 5 days. Also, cells pretreated with 2 μM $HgCl_2$, 1 μM $CdCl_2$, or 40 μM $ZnCl_2$ for 20 h were activated with LPS (40 μg ml^{-1}) for 30 min; 25,000 of these cells were suspended in 1 ml of complete medium and assessed for growth to determine the effect of metal pretreatment plus LPS on viability.

Metabolic assay THP-1 cells, 1×10^5 per well in 100 μl complete medium were seeded in 96-well flat-bottomed tissue culture plates (Nunc, BRL) for 4 h before addition of 10 μl of zinc, cadmium or mercury salts (prepared at high concentration in RPMI minus Fetaclone) to produce the metal concentrations described above. After 3 days of growth, 10 μl alamarBlue reagent was added, and absorbance at A_{595} was measured with a BioRad Model 3550 microplate reader. Cellular metabolic activity results in the reduction of alamarBlue with a concomitant colorimetric change. Control cells were assessed for viability identically, except that medium without metals was added. Relative cell survival for drug treatments was expressed as a fraction of viability in cells untreated with metal salts.

The toxicity of LPS induction, or zinc, mercury, or cadmium salts followed by LPS induction, was assessed by trypan blue dye exclusion.

Measurement of cellular Hg^{2+} association

$^{203}Hg^{2+}$ as a mercuric ion (Hg^{2+}) (2.75×10^6 d.p.m. μg^{-1}) (Buffalo Materials Corp., Buffalo, NY) was added to non-radioactive $HgCl_2$ in RPMI 1640 + 10% Fetaclone to produce 2 μM Hg^{2+} emitting approximately 200,000 d.p.m. ml^{-1}. One ml of this solution was added to triplicate aliquots of 1×10^6 THP-1 cells pelleted by centrifugation in 1.5 ml polypropylene microcentrifuge tubes (1000 r.p.m. for 1 min). At 0, 8.75, 20.5, 35.9, 65.75, and 240 min, cells in 3 microcentrifuge tubes were precipitated by centrifugation and the supernatants removed to fresh tubes, the cell pellets were washed with 500 μl each of phosphate-buffered saline (PBS) and the PBS wash added to each supernatant. The recorded time point is the time at which the wash buffer was added to the centrifuged cell pellet. Triplicate samples of complete medium without cells were processed at the same time to determine the total recoverable radioactivity in the absence of cells. By use of the known specific activity of added Hg^{2+}, results were reduced to 'Hg associated with cells (pmol per 1×10^6 cells)', and 'Hg remaining in medium (pmol)' for each time point.

Measurement of respiratory burst

H_2O_2 production The functional activation of cells was measured by a modification of the phenol red assay (Pick & Keisari, 1980) which evaluates the activated state of cells by measuring the horseradish peroxidase-mediated oxidation of phenol red by H_2O_2 (the conversion product of superoxide anions). Briefly, 1×10^6 THP-1 cells were precipitated in 1.5 ml polypropylene microfuge tubes (GIBCO/BRL) and induced with LPS as described above. Control cells had no LPS added. Thirty min after addition of LPS, cells were precipitated by centrifugation and the supernatant discarded. One ml of phenol red solution (PRS: Hanks' balanced salt solution containing 0.28 mM phenol red (Sigma), and 8.5 units ml^{-1} horseradish peroxidase (Sigma) was added to the cell pellet and the cells dispersed by gentle vortexing. After 1 h in PRS, cells were precipitated by centrifugation and the 1 ml supernatant transferred to a new microcentrifuge tube and made alkaline by the addition of 10 μl 1 M NaOH. The absorbance of the resulting coloured product was measured on a Beckman spectrophotometer at 610 nm. Sample concentrations of H_2O_2 were estimated from a standard curve generated by measuring the absorbance of a range of H_2O_2 concentrations at 610 nm.

Measurement of adhesion

THP-1 cells (3×10^6) in 3 ml of medium plus FBS were seeded into the wells of 6 well plates and incubated for 16 h with indicated concentrations of Zn^{2+}, Cd^{2+} or Hg^{2+} salts. Control cells received no added metal salts. All cells were treated with

bacterial LPS (40 μg ml^{-1}) as described above and the wells washed twice with 5 ml PBS at the end of the 30 min incubation period. One ml of 0.1% sodium dodecyl sulphate (SDS) was added to each well to solubilize cells, incubated 10 min at 37°C, and A_{260} measured as an indicator of the number of cells adhering to the plate. Measured absorbance for untreated control cells was normalised to 1.0.

Measurement of MT-2 and IL-1β mRNA

THP-1 cells pretreated for 20 h without metals, or with Zn^{2+}, CD^{2+} or Hg^{2+} salts ± subsequent LPS induction, were flash-frozen in liquid nitrogen and total cellular RNA was isolated by use of modification of the guanidinium isothiocyanate-phenol-chloroform method (Sambrook *et al*., 1989). Ten μg of total RNA was separated by electrophoresis through a denaturing formaldehyde gel and transferred to Hybond-N nylon paper (Amersham Canada, Oakville, Ontario, Canada) as described previously (Koropatnick *et al*., 1983). Northern blots were hybridized, in sequence, to cDNA probes specific for human MT-2 mRNA (a BamH1/PvuII 170 bp fragment, the kind gift of Dr M. Karin), human IL-1β mRNA (a 1.05 kb sequence (March *et al*., 1985)), and 18S rRNA (Behrend *et al*., 1994) by the method of Church and Gilbert (1984). Briefly, blots were hybridized in a solution containing 1 mM EDTA, 0.5 M $NaHPO_4$, pH 7.2, and 7% sodium lauryl sulphate (SLS) at 65°C for 16 h. The filters were washed in posthybridization buffer (1 mM sodium EDTA, 40 mM $NaHPO_4$, pH 7.2, and 1% SLS) twice for 10 min each at 65°C and were exposed to a phosphor screen. MT-2 mRNA, IL-1β mRNA, and 18S rRNA were quantitated densitometrically by a Phosphorimager and the ImageQuant programme (Molecular Dynamics, Sunnyvale, CA). Relative mRNA levels were defined as (MT-2 or IL-1β signal)/(18S rRNA signal) in the same sample of RNA.

Measurement of MT protein

Cells were lysed by three cycles of freeze-thawing in liquid N_2 and a 65°C water bath, incubated at 70°C for 10 min, and then cooled for 10 min on ice. Heat-precipitable proteins were removed from the sample by centrifugation at $5{,}000 \times g$ for 20 min. MT protein concentrations in supernatants were estimated, in triplicate, by use of a rabbit antiserum to rat MT-2 (which has equal affinity to human MT-1 and MT-2 isoforms) and a competitive ELISA for MT (Leibbrandt *et al*., 1991). The amount of MT protein was expressed relative to total soluble cellular protein (estimated by a modification of the method of Bradford (1976).

Statistical analysis

All values presented represent mean ± s.e. Differences between means for corresponding sets of data were evaluated statistically by a 1-way analysis of variance (ANOVA) followed by Tukey's protected *t* test, which is a *post hoc* multiple comparison test. Data expressed as a fraction or % were first normalized before any parametric statistical procedure was implemented. This normalization was accomplished by applying the arcsine transformation, which takes the arcsine of the square root of the decimal fraction of the % or fractional score. The level of significance for all statistical analyses was chosen *a priori* to be $P < 0.05$.

Results

Hg^{2+}, Cd^{2+} and Zn^{2+} toxicity to THP-1 cells

In order to assess changes in the behaviour of human monocytes in response to LPS caused by exposure to low, non-toxic exposure to metals, we measured cellular metabolic activity (by MTT assay) (Figure 1) and proliferation potential (Figure 2), in cells continuously treated with added Hg^{2+}, Cd^{2+} and Zn^{2+} salts for 4 to 5 days. Hg^{2+} had no significant effect on either parameter at concentrations up to 25 μM, but severely inhibited metabolism at 50 μM. Up to 5 μM, cadmium had no significant effect on metabolic activity, and 1 μM cadmium had no effect on proliferation. Ten μM cadmium significantly decreased metabolic activity and proliferation. Added zinc chloride at both 20 and 40 μM had no significant effect on either metabolic activity or proliferation.

Hg^{2+} association with THP-1 cells

To determine the kinetics of Hg^{2+} association with THP-1 cells over the time of MT mRNA induction, we measured radio-

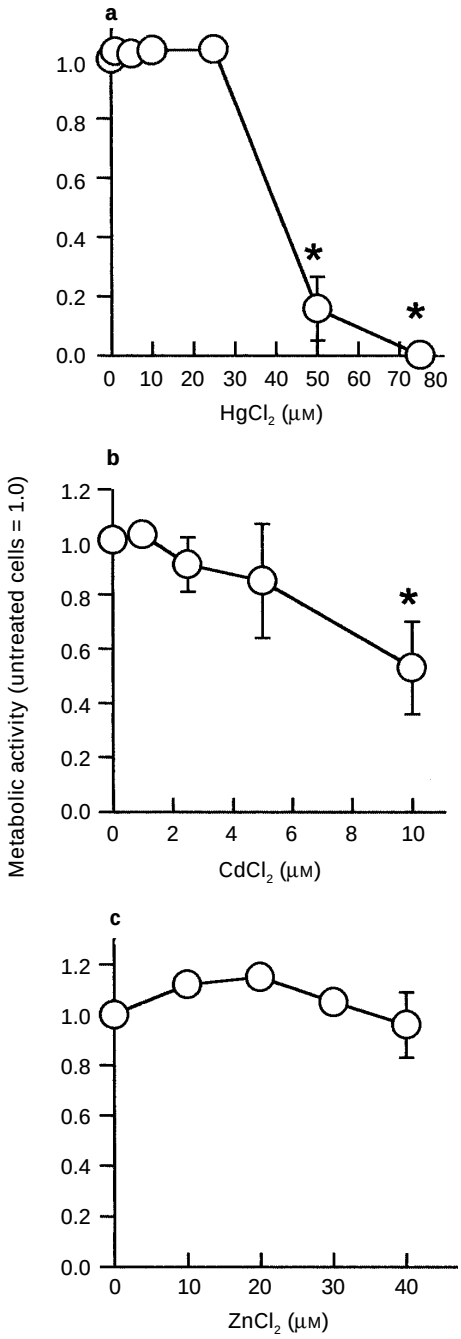

Figure 1 Metabolic inhibition of THP-1 cells treated with added (a) $HgCl_2$, (b) $CdCl_2$ or (c) $ZnCl_2$; 1×10^5 cells per well of a 96 well plate were exposed to the indicated added metal salt concentrations, and metabolic activity assessed, as described in Methods section. Data points indicate the mean ± s.d. from 4 independent measurements. Where error bars do not appear, they are smaller than the size of the symbol. *Significantly different from mean values for untreated cells.

active mercury uptake from 0 to 4 h in cells exposed to 2 μM $^{203}HgCl_2$ (Figure 3). Hg^{2+} associated with cells increased rapidly over the first 75 min of exposure. Approximately 2–2.5% of administered Hg^{2+} in bathing medium becoming associated with precipitated cells by that time, indicating that Hg^{2+} was either bound to membrane components or within THP-1 cells. Over 4 h, there was no significant change in Hg^{2+} concentration in the extracellular medium, indicating that cells were exposed to the same concentration of Hg^{2+} over the course of the experiment.

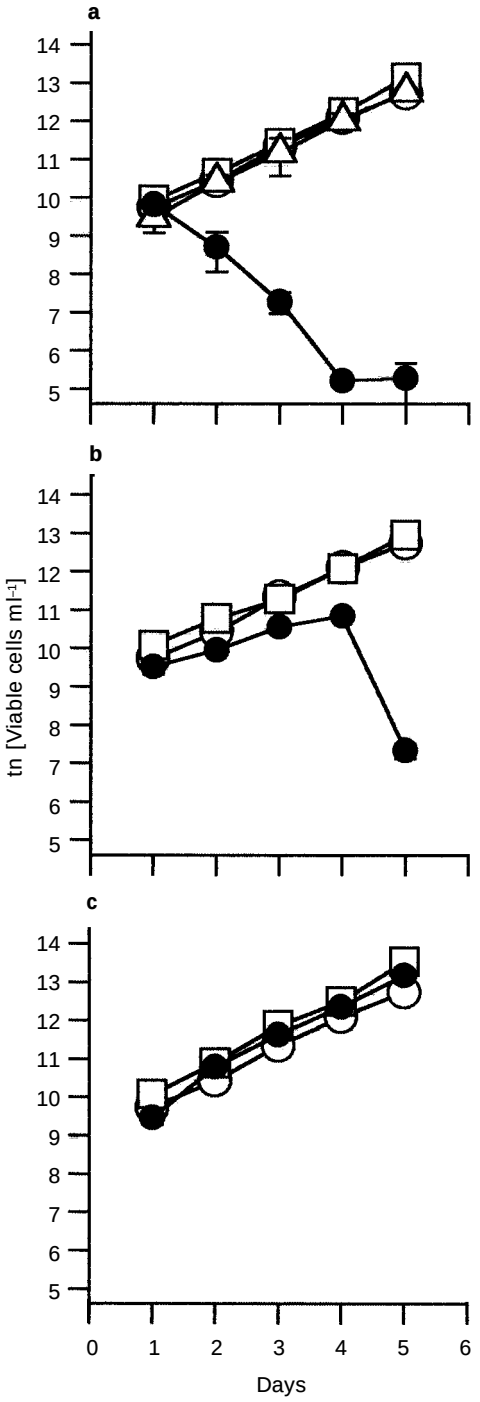

Figure 2 Proliferation of THP-1 cells treated with added (a) $HgCl_2$, (b) $CdCl_2$, or (c) $ZnCl_2$. Cells were exposed to added metal salt concentrations as described in Figure 1; 1×10^4 cells from each treatment group were added to separate wells of a 20 well tissue culture plate, in 2 ml of medium, and the number counted on successive days for 5 days. Symbols represent the mean $\pm$ s.d. of 3 independent measurements: where error bars do not appear, they are smaller than the size of the symbol. (a) $HgCl_2$: (○) control, (□) 2 μM Hg^{2+}, (△) 20 μM Hg^{2+} and (●) 50 μM Hg^{2+}; (b) $CdCl_2$: (○) control, (□) 1 μM Cd^{2+} and (●) 10 μM Cd^{2+}; (c) $ZnCl_2$: (○) control, (□) 20 μM Zn^{2+} and (●) 40 μM Zn^{2+}.

Induction of MT, but not IL-1β, by metal salt pretreatment

Mercury, zinc and cadmium salts all induce MT in mammalian cells *in vitro* (Durnam & Palmiter, 1984; Koizumi *et al*., 1993) and in organs of whole animals (Saijoh *et al*., 1989). We tested the ability of non-toxic treatments with Hg^{2+}, Zn^{2+} and Cd^{2+} salts to induce MT-2 mRNA (Figures 4 and 5) and MT protein (Figure 6) in THP-1 cells. Hg^{2+} treatment, 2 and 10 μM for 20 h, increased MT-2 mRNA and MT protein accumulation approximately two fold. Zn^{2+}, 20 and 40 μM caused an increase in cellular content of MT-2 mRNA two fold and ten fold, respectively; MT protein content was increased three fold and six fold, respectively. Cd^{2+}, 1 μM, induced a fourteen fold increase in MT-2 mRNA and an eight fold increase in MT protein. Cd^{2+}, 10 μM which induced significant toxicity (Figures 1 and 2), also induced significantly less MT-2 mRNA than 1 μM Cd^{2+} (Figure 4), and did not induce a significantly higher level of MT protein than that induced by 1 μM Cd^{2+} (Figure 6). None of the metal treatments had any effect on IL-1β

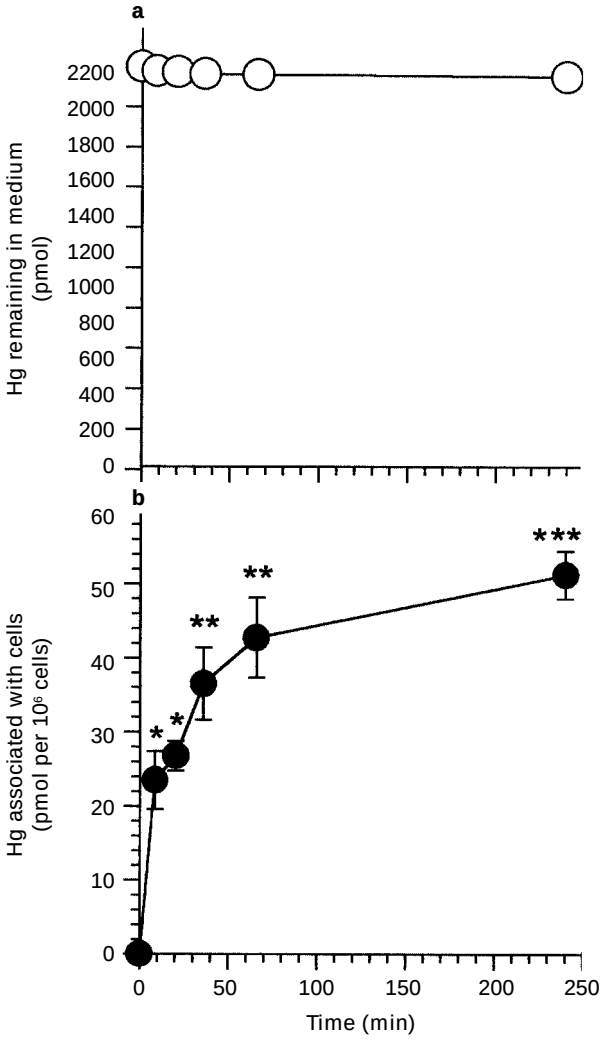

Figure 3 $HgCl_2$ association with THP-1 cells. THP-1 cells 1×10^6 were treated with $^{203}HgCl_2$ 2 μM for up to 4 h and $^{203}Hg^{2+}$ associated with (b) cells or (a) supernatant assessed as described in Methods section. Symbols represent the mean of 3 independent measurements; vertical lines show s.e. *Significantly different from mean values for cells untreated with Hg^{2+}; **significantly different from mean values of cells untreated with Hg^{2+}, but not from cells sampled approximately 60 min after addition of Hg^{2+}.

mRNA levels (Figures 4 and 5). Thus, in human monocytes treated with non-toxic concentrations of metal salts, Hg^{2+}, Cd^{2+} and Zn^{2+} all caused a significant increase in MT mRNA and protein levels by 20 h of treatment in the following order of effectiveness: $Cd^{2+} > Hg^{2+} > Zn^{2+}$.

Suppression of MT-2 and IL-1β mRNA induction, and respiratory burst and increased adhesion by metal salt pretreatment

Bacterial lipopolysaccharide treatment induces activation in human monocytes. Markers of activation include increased production and release of toxic oxygen metabolites, lysosomal hydrolases, and colony-stimulating factor (Pabst & Johnston, 1980) and increased adhesion to plastic (Auwerx *et al*., 1989). Increased transcription, synthesis and secretion of metalloproteinases, IL-1α and β, interferon γ, and various arachidonic acid metabolites *in vitro* (Koj, 1985) accompany activation. Metallothionein-2 (MT-2) is also synthesised by activated monocytes (Leibbrandt & Koropatnick, 1994). We measured LPS-induced production of superoxide radicals (as indicated by hydrogen peroxide accumulation in the phenol red assay), IL-1β mRNA, MT-2 mRNA and MT protein, and increased adhesion to plastic tissue culture plates, as parameters of activation in THP-1 cells, pretreated or not pretreated with metal salts at low, non-toxic concentrations. Treatment of THP-1 cells with LPS (40 μg ml^{-1}) alone induced an approximately eight fold increase in hydrogen peroxide level within 1 h (Figure 7). Lower concentrations of LPS (1 μg ml^{-1} or 10 μg ml^{-1}) induced approximately three fold and five fold

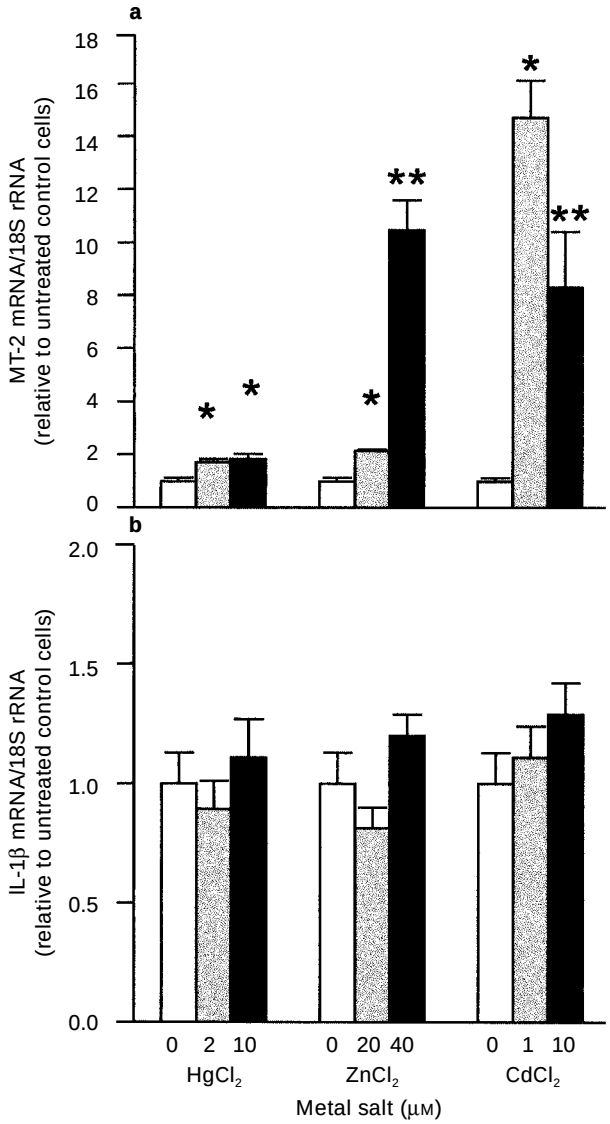

Figure 4 Nontoxic exposure to added Hg^{2+}, Zn^{2+} and Cd^{2+} salts induced metallothionein-2 (MT-2) mRNA (a), but not interleukin-1β (IL-1β) mRNA (b), in THP-1 cells. Triplicate samples of THP-1 cells were independently exposed to the indicated added metal salt concentrations for 20 h, and assessed for hMT-2, hIL-1β, and 18S rRNA levels in the same lanes as described in Methods section. MT-2 and IL-1β mRNA are presented as the level relative to 18S rRNA. Plotted values are the mean of 3 measurements ± s.d. relative to the value for untreated control cells, for which values were normalized to 1.0. *Significantly different from mean values for untreated control cells; **significantly different from mean values for untreated control cells, and cells treated with a lower Zn^{2+} or Cd^{2+} concentration.

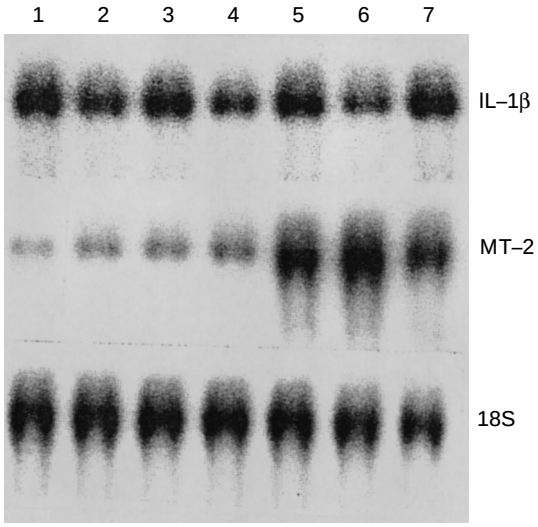

Figure 5 Representative Northern blot of data presented in Figure 4. THP-1 cells were treated for 20 h with, or without (control cells), Hg^{2+}, Zn^{2+} or Cd^{2+} salts, and assessed for IL-1β and MT-2 mRNA. 18S rRNA blots are shown to indicate comparative RNA loading in each lane. Lane 1: Control cells, lane 2: 2 μM Hg^{2+}, lane 3: 10 μM Hg^{2+}, lane 4: 20 μM Zn^{2+}, lane 5: 40 μM Zn^{2+}, lane 6: 1 μM Cd^{2+}, lane 7: 10 μM Cd^{2+}.

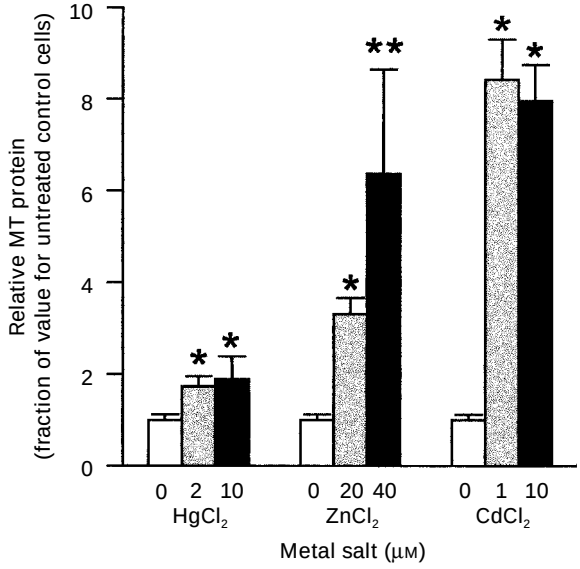

Figure 6 Nontoxic treatment with added Hg^{2+}, Zn^{2+} and Cd^{2+} salts induced MT protein in THP-1 cells. Samples of the same cells described in the legend to Figure 4 were assessed for MT protein by a competitive radioimmunoassay as described in Methods section. *Significantly different from mean values for untreated control cells; **Significantly different from mean values for untreated control cells, and cells treated with a lower Zn^{2+} concentration.

increases, respectively, in hydrogen peroxide levels (data not shown). Concentrations lower than 1 μg ml^{-1} induced no detectable increase in hydrogen peroxide (data not shown). LPS concentrations (1 to 10 μg ml^{-1}) have been used to induce activation in THP-1 cells (Leibbrandt *et al*., 1994; Leibbrandt & Koropatnick, 1994; Sanceau *et al*., 1995; Groupp & Donovan-Peluso, 1996). However, the maximal induction of respiratory burst by 40 μg ml^{-1} LPS, and the fact that 40 μg ml^{-1} LPS had no discernible toxic effect on THP-1 cells, as assessed by trypan blue exclusion (data not shown), led us to choose the higher concentration to induce activation. Pretreatment with 20 μM Zn^{2+}, 1 μM Cd^{2+} or 2 μM Hg^{2+} for 20 h reduced LPS-stimulated oxygen radical production by more than 300%, greater than 500% and 200%, respectively (Figure 7). Although LPS induction alone decreased proliferation by inducing differentiation into the non-dividing macrophage line, metal salt pretreatment did not further decrease the ability of THP-1 cells to proliferate over a 5 day period (data not shown). In addition, LPS treatment, with or without metal pretreatment, did not diminish the capacity of THP-1 cells to exclude trypan blue dye. Taken together, these data indicate that combined metal salt and LPS treatment did not synergistically induce toxic events. Hg^{2+} 2 μM, Cd^{2+} 1 μM and Zn^{2+} 20 and 40 μM had no ability, on their own, to induce or suppress oxygen radical production in THP-1 monocytes (data not shown).

With respect to LPS-induced IL-1β and MT-2 mRNA levels (both of which indicate activation), pretreatment with Zn^{2+}, Cd^{2+} or Hg^{2+} significantly depressed induction of both mRNAs (Figures 8 and 9). LPS-induced IL-1β mRNA levels were more acutely decreased (200–300%) than MT-2 mRNA levels (25–50%). In addition, pretreatment with all metal salts significantly inhibited LPS-induced enhancement in THP-1 cell adhesion to tissue culture plates (Figure 10): in the case of $HgCl_2$, concentrations as low as 0.2 μM Hg^{2+} were effective. Therefore, exposure of human monocytes to non-toxic concentrations of the non-essential metals Hg^{2+} and Cd^{2+} had an inhibitory effect on markers of activation (MT-2 mRNA and protein, IL-1β mRNA, and oxygen radical upregulation), similar to the inhibitory effect of the essential metal zinc on LPS-induced respiratory burst and MT mRNA and protein production (Leibbrandt & Koropatnick, 1994), and on increased adhesion, which is a marker of both activation and differentiation in the macrophage line (Auwerx *et al*., 1989).

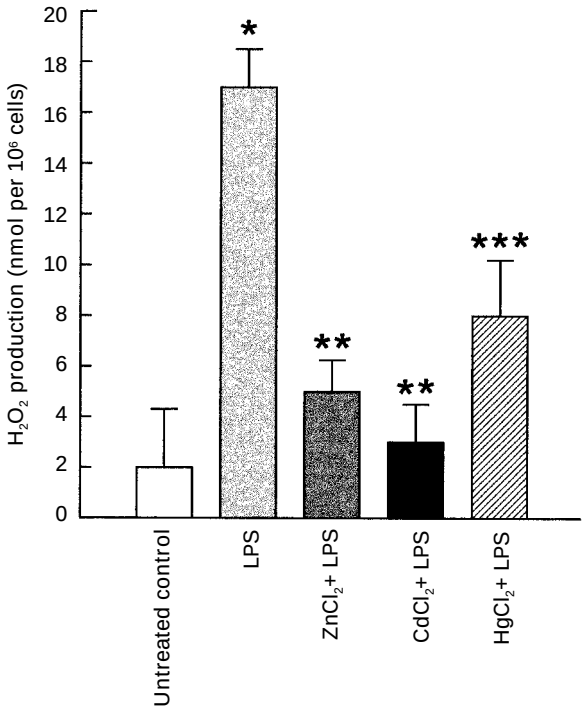

Figure 7 Respiratory burst activity induced in THP-1 cells by LPS is diminished by non-toxic treatment with added Zn^{2+}, Cd^{2+} or Hg^{2+} salts. Cells were exposed for 20 h to 20 μM $ZnCl_2$, 1 μM $CdCl_2$ or 2 μM $HgCl_2$, then induced for 1 h with LPS, and assessed for production of oxygen free radicals as described in Methods section. The mean of 5 independent measurements $\pm$ s.d. is plotted for each column. *Significantly different from mean values for untreated control cells; **significantly different from mean values for cells treated with LPS alone; ***significantly different from mean values for cells treated with LPS alone, and cells pretreated with Zn^{2+} or Cd^{2+} salts before LPS induction.

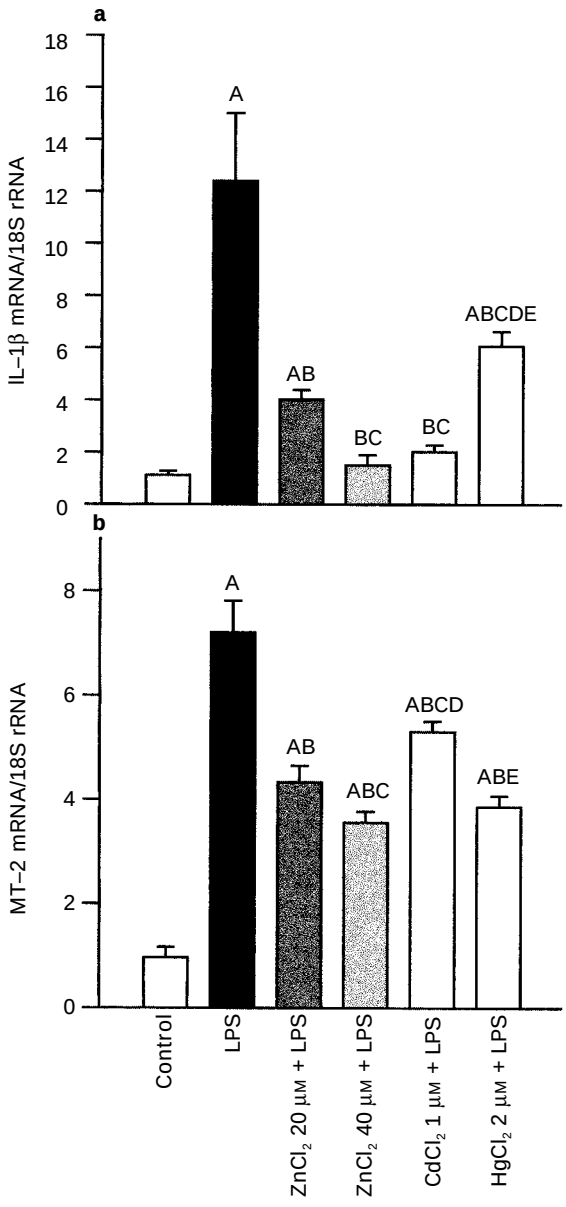

Figure 8 (a) Interleukin-1β (IL-1β) and (b) metallothionein-2 (MT-2) mRNA induction by LPS treatment of THP-1 cells is diminished by nontoxic 20 h pretreatment with 20 or 40 μM $ZnCl_2$, 1 μM $CdCl_2$ or 2 μM $HgCl_2$. Metal salt and LPS treatments, and MT-2, IL-1β, and 18S rRNA measurements, were performed as described in Methods section. Plotted values are the mean of 3 measurements $\pm$ s.d. relative to the value for untreated control cells, for which values were normalized to 1.0. [A]Significantly different from mean values for untreated control cells; [B] significant different from mean values for LPS-treated cells; [C]significantly different from mean values for 20 μM Zn^{2+} + LPS-treated cells; [D]significantly different from mean values for 40 μM Zn^{2+} + LPS-treated cells; [E]significantly different from mean values for 1 μM $CdCl_2$ + LPS-treated cells.

Discussion

We assessed the ability of low, non-toxic concentrations of Cd^{2+} and Hg^{2+} to modulate activation of human monocytes *in vitro*. We demonstrated previously that non-toxic pretreatment of monocytic leukaemia THP-1 cells with zinc depressed LPS-induced activation (Leibbrandt & Koropatnick, 1994), in spite of the fact that most effects of zinc treatment appear to stimulate mononuclear cell functions (Keen & Gershwin, 1990). As with zinc, low levels of both cadmium and mercury inhibited not only oxygen radical production, but cytokine production (IL-1β) normally induced by LPS. Interestingly, MT-2 mRNA and protein induction, normally markedly induced by LPS treatment, were significantly inhibited by cadmium and mercury pretreatment, similar to the inhibition observed after zinc exposure. Antisense RNA-mediated specific inhibition of MT expression abolishes LPS-induced activation (Leibbrandt *et al*., 1994), indicating a critical role for MT in monocyte activation. In view of this role, it is possible that LPS-induced repression of MT following metal induction may be a causal event in the lower level of activation observed after metal induction of MT expression.

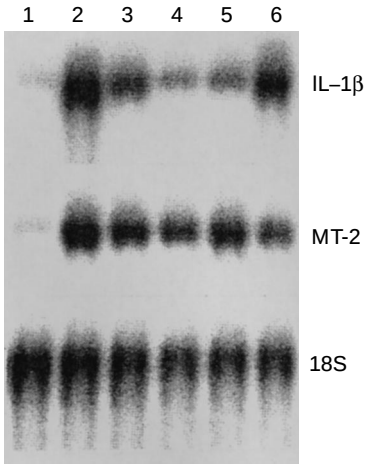

Figure 9 Representative Northern blot of data presented in Figure 8. THP-1 cells were treated for 20 h with, or without (control cells), Hg^{2+}, Zn^{2+} or Cd^{2+} salts, activated with bacterial LPS, and assessed for hIL-1β and hMT-2 mRNA. 18S rRNA blots are shown to indicate comparative RNA loading in each lane. Lane 1: control cells minus LPS induction; lane 2: control cells plus LPS induction, lane 3: 20 μM Zn^{2+}-pretreated cells plus LPS induction; lane 4: 40 μM Zn^{2+}-pretreated cells plus LPS induction; lane 5: 1 μM Cd^{2+}-pretreated cells plus LPS induction; lane 6: 2 μM Hg^{2+}-pretreated cells plus LPS induction.

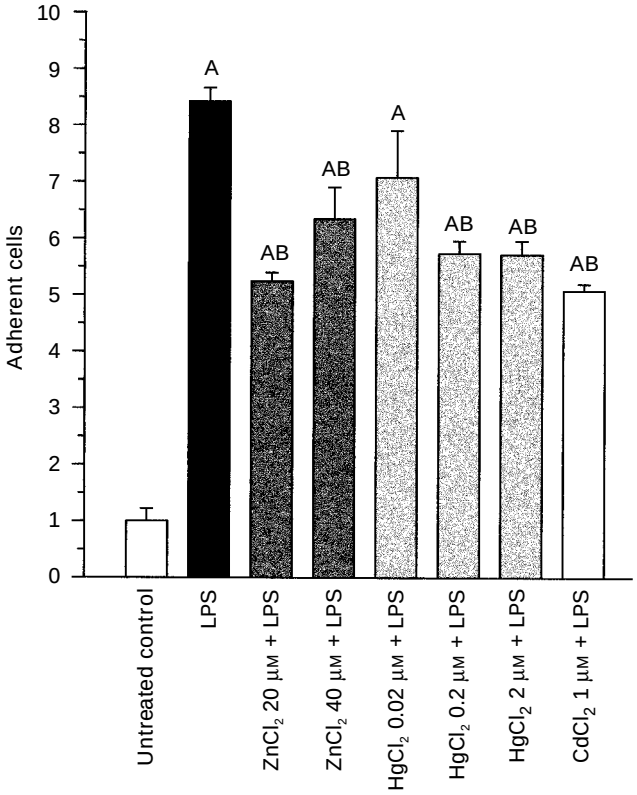

Figure 10 Enhanced adhesion induced in THP-1 cells by LPS is diminished by non-toxic treatment with Zn^{2+}, Cd^{2+} or Hg^{2+} salts. THP-1 cells, 3×10^6 were treated for 45 min with LPS (40 μg ml^{-1}) and adherent cells measured as described in Methods section. [A]Significantly different from untreated control; [B]significantly different from cells treated with LPS alone.

The lower level of production of MT, IL-1β and oxygen radicals following combined zinc and LPS treatment did not result from a synergistic effect of metal plus LPS to induce cellular toxicity. Cells treated with Hg^{2+}, CD^{2+} or Zn^{2+} plus LPS continued to proliferate at least as well as cells treated with LPS alone. In fact, cells exposed to metals followed by LPS had a slightly higher proliferation potential (data not shown) and less propensity to attach themselves to tissue culture plates (Figure 10) than cells induced with LPS alone. Since activation stimulates terminal differentiation in macrophages (characterized by increased cellular adherence and decreased proliferation (Auwerx *et al*., 1989)) metal pretreatment-induced decreases in these effects may be a further indication of decreased activation.

Zn^{2+} or Cd^{2+} treatment alone (without subsequent LPS activation) increased MT-2 mRNA ten to fourteen fold (Figure 4). LPS induction of metal-pretreated cells induced a decrease in MT-2 mRNA to levels four to five fold higher than control cells (Figure 7). This is in agreement with our previous observation of rapid (within 15 min), time-dependent downregulation of both MT mRNA and MT protein levels in response to LPS in zinc-pretreated monocytes (Leibbrandt & Koropatnick, 1994). The mechanism by which downregulation occurs remains to be elucidated. However, the short time-period within which it occurs suggests to us that LPS-induced MT mRNA and protein degradation, rather than inhibition of MT gene transcription, may be the cause (Leibbrandt *et al*., 1994).

The mechanism by which these metals affect LPS-induced activation is not known. Evidence suggests that LPS signals activation through interactions with an LPS-binding protein that associates with the CD14 glycoprotein in the monocyte plasma membrane. Higher LPS concentrations may activate through a mechanism not mediated by CD14 (Haziot *et al*., 1996). Interestingly, we have seen that activation of THP-1 cells by phorbol myristate acetate is inhibited by Hg^{2+}, Cd^{2+} and Zn^{2+} pretreatment (manuscript in preparation). Phorbol myristate acetate-induced activation is independent of CD14, but, like LPS, proceeds through a protein kinase C-mediated pathway (Nishizuka, 1992). This suggests that metal-mediated suppression of activation occurs through a mechanism that alters events within or following protein kinase C signalling, rather than affecting the function of CD14.

We have previously shown that downregulation of MT in THP-1 cells abolishes LPS-induced activation (Leibbrandt *et al*., 1994). It would be reasonable to expect that metal pretreatment to induce MT would enhance rather than inhibit activation. However, we observed the opposite. Although the data presented here do not resolve the issue, we believe that at least two hypotheses may explain the observations. First, MT levels might need to be in a relatively narrow range in order to mediate induction of activation: either increasing or decreasing those levels would have a negative effect. Second, metal treatment can affect the expression of other genes in addition to those encoding MT. In view of this latter possibility, non-

MT factors induced by metals might act independently, or in conjunction with MT, to inhibit activation potential. We are transfecting monocytes with expression vectors designed to increase the intracellular concentration of MT specifically in a stepwise manner without treatment with exogenous metals. Assessment of the activation potential of these cells will test these hypotheses.

Zinc is known to stabilize plasma and lysosomal membranes (Bettger & O'Dell, 1981). Cd^{2+} (Muller & Menzel, 1990) and Hg^{2+} (Benov *et al*., 1990; Rungby & Ernst, 1992) induce events that lead to lipid peroxidation in cellular membranes. It is possible that mild peroxidation events that do not lead to cellular toxicity nevertheless interfere with LPS interactions important in signal transduction. In addition, Hg^{2+}, Zn^{2+} and Cd^{2+} all interact with metallothionein: considering the important role that MT plays in activating monocytes (Leibbrandt *et al*., 1994), metal ion/MT interactions may directly affect MT activities critical to monocyte activation or MT protein stability. The presence of the metal ions alone can also induce MT gene transcription. Altered MT gene transcription, stability, and/or function as a result of the presence of low levels of Hg^{2+}, Zn^{2+} or Cd^{2+} could lead to diminished response to LPS. MT has been suggested to control homeostatically availability of zinc to proteins important in signalling or mediating activation (Kagi & Schaffer, 1988; Zeng *et al*., 1991; O'Halloran, 1993; Leibbrandt *et al*., 1994) and can bind and inactivate reactive oxygen species (Tamai *et al*., 1993; Chubatsu & Meneghini, 1993) important in regulating eukaryotic transcription factors (Angel *et al*., 1987; Devary *et al*., 1991; Alam & Smith, 1992; Gralla & Kosman, 1992). In this capacity, altered MT expression and/or function could have profound effects on the ability of LPS to activate cells, or for monocytes to carry out activation-associated events.

The non-toxic concentrations of Zn^{2+} capable of inhibiting monocyte activation (20–40 μM) are close to the physiological range of 13–17 μM in human blood (Goyer, 1991), raising the possibility that physiological changes in plasma Zn^{2+} concentration may have consequences for human white blood cell function *in vivo*. Whether the inhibitory concentrations of Cd^{2+} or Hg^{2+} are also within plasma concentration ranges achieved in man or animals exposed to these metal ions is more difficult to assess, as the very rapid clearance of these metals from plasma after both acute and chronic exposure makes measurement of metal concentrations within the plasma compartment highly variable (Friberg & Elinder, 1992). Nevertheless, Cd^{2+} and Hg^{2+} concentrations that inhibit activation were clearly ten to twenty fold lower than the minimum concentration required to induce measurable toxicity.

In summary, low-level pretreatment of human monocytes with Hg^{2+}, Cd^{2+} or Zn^{2+} salts profoundly decreases the ability of these cells to respond to activation by bacterial LPS. Although metals at these low concentrations exert no detectable toxicity, they are capable of transmitting transcriptional signals, since MT-2 mRNA and MT protein are induced by all metal treatments. Subsequent LPS induction diminishes MT mRNA and protein levels, in contrast to the upregulation in MT expression associated with activation of monocytes not pre-exposed to metals. Depression of monocyte activation (an anti-inflammatory effect) may be an important consequence of chronic, low-level exposure to both essential and non-essential metals at levels below those that induce measurable cytotoxicity or decreased proliferation potential.

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References

ALAM, J. & SMITH, A. (1992). Heme-hemopexin-mediated induction of metallothionein gene expression. *J. Biol. Chem*., **267,** 16379–16384.

ANDREWS, G.K. (1990). Regulation of metallothionein gene expression. *Prog. Food Nutr. Sci*., **14,** 193–258.

ANGEL, P., IMAGAWA, M., CHIU, R., STEIN, B., IMBRA, R.J., RAHMSDORF, H.J., JONAT, C., HERRLICH, P. & KARIN, M. (1987). Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. *Cell*, **49,** 729–739.

ATEN, J., BOSMAN, C.B., ROZING, J., STIJNEN, T., HOEDEMAEKER, P.J. & WEENING, J.J. (1988). Mercuric-chloride-induced autoimmunity in the Brown Norway rat: Cellular kinetics and major histocompatibility complex antigen expression. *Am. J. Pathol*., **133,** 127–138.

AUWERX, J.H., CHAIT, A., WOLFBAUER, G. & DEEB, S.S. (1989). Loss of copper-zinc superoxide gene expression in differentiated cells of myelo-monocytic origin. *Blood*, **74,** 1807–1810.

BEHREND, E.I., CRAIG, A.M., WILSON, S.M., DENHARDT, D.T. & CHAMBERS, A.F. (1994). Reduced malignancy of ras-transformed NIH 3T3 cells expressing antisense osteopontin RNA. *Cancer Res*., **54,** 832–837.

BENOV, L.C., BENCHEV, I.C. & MONOVICH, O.H. (1990). Thiol antidotes effect on lipid peroxidation in mercury-poisoned rats. *Chem.-Biol. Interact*., **76,** 321–332.

BETTGER, W. & O'DELL, B.L. (1981). A critical physiological role of zinc in the structure and function of biomembranes. *Life Sci*., **28,** 1425–1438.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72,** 279–291.

BRIGGS, W.A., PEDERSEN, M.M., MAHAJAN, S.K., SILLIX, D.H., PRASAD, A.S. & McDONALD, F.D. (1982). Lymphocyte and granulocyte function in zinc-treated and zinc-deficient hemodialysis patients. *Kidney Int*., **21,** 827–832.

CASTILLO-DURAN, C., HERESI, G., FISBERG, M. & UAUY, R. (1987). Controlled trial of zinc supplementation during recovery from malnutrition: Effects on growth and immune function. *Am. J. Clin. Nutr*., **45,** 602–608.

CHOWDHURY, B.A. & CHANDRA, R.K. (1989). Effect of zinc administration on cadmium-induced suppression of natural killer cell activity in mice. *Immunol. Lett*., **22,** 287–291.

CHURCH, G.M. & GILBERT, W. (1984). Genomic sequencing. *Proc. Natl. Acad. Sci. U.S.A*., **81,** 1991–1995.

CHUBATSU, L.S. & MENEGHINI, R. (1993). Metallothionein protects DNA from oxidative damage. *Biochem. J*., **291,** 193–198.

CHVAPIL, M., STANKOVA, L., ZUKOSKI IV, C. & ZUKOSKI III, C. (1977). Inhibition of some functions of polymorphonuclear leukocytes by in vitro zinc. *J. Lab. Clin. Med*., **89,** 135–141.

CIFONE, M.G., ALESSE, E., DIEUGENIO, R., NAPOLITANO, T., MORRONE, S., PAOLINI, R., SANTONI, G. & SANTONI, A. (1989). In vivo cadmium treatment alters natural killer activity and large granular lymphocyte number in the rat. *Immunopharmacology*, **18,** 149–156.

DEVARY, Y., GOTTLIEB, R.A., LAU, L.F. & KARIN, M. (1991). Rapid and preferential activation of the c-jun gene during the mammalian UV response. *Mol. Cell. Biol*., **11,** 2804–2811.

DURNAM, D.M. & PALMITER, R.D. (1984). Induction of metallothihonein-1 mRNA in cultured cells by heavy metals and iodoacetate: Evidence for gratuitous inducers. *Mol. Cell. Biol*., **4,** 484–491.

FABRIS, N., MOCCHEGIANI, E., GALLI, M., IRATO, L., LAZZARIN, A. & MORRONI, M. (1988). AIDS, zinc deviciency and thymic hormone failure. *J. Am. Med. Assoc*., **259,** 839–840.

FRIBERG, L. & ELINDER, C.G. (1992). Cadmium. IPCS Environmental Health Criteria 134, World Health Organization, Geneva, pp. 18–20.

FRIBERG, L. & LENER, J. (1986). Molybdenum. In *Handbook on the Toxicology of Metals*, Vol. II, 2nd ed, *Specific Metals* ed. Friberg, L., Nordberg, G.F. & Vouk, V.B. Amsterdam: Elsevier Scientific.

GOYER, R.A. (1991). Toxic effects of metals. In: Toxicology – The basic science of poisons (Fourth Ed.). ed. Amdur, M.O., Doull, J. & Klaassen, C.D. pp. 623–680. New York: Pergamon Press.

GRALLA, E.B. & KOSMAN, D.J. (1992). Molecular genetics of superoxide dismutases in yeasts and related fungi. *Adv. Genet.*, **30,** 251–319.

GROUPP, E.R. & DONOVAN-PELUSO, M. (1996). Lipopolysaccharide induction of THP-1 cells activates binding of c-jun, Ets, and Egr-1 to the tissue factor promoter. *J. Biol. Chem.*, **271,** 12423–12430.

HAZIOT, A., FERRERO, E., KONTGEN, F., HIJIYA, N., YAMAMOTO, S., SILVER, J., STEWART, C.L. & GOYERT, S.M. (1996). Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. *Immunity*, **4,** 407–414.

HERTEL, R.F. & WIBBERTMANN, A. (1990). Adverse effects of zinc on the immune system. In *Immunotoxicity of Metals and Immunotoxicology*. ed. Dayan, A.D., Hertel, R.F., Heseltine, E., Kazantzis, G., Smith, E.M. & van der Venne, M.T. pp. 223–233. New York: Plenum.

HULTMAN, P., BELL, L.J., ENESTROM, S. & POLLARD, K.M. (1993). Murine susceptibility to mercury. II. Autoantibody profiles and renal immune deposits in hybrid, backcross, and H-2^d congenic mice. *Clin. Immunol. Immunopathol.*, **68,** 9–20.

KAGI, J.H.R. & SCHAFFER, A. (1988). Biochemistry of metallothionein. *Biochemistry*, **27,** 8509–8515.

KAZIMIERCZAK, W. & MASLINSKI, C. (1974). Histamine release from mast cells by compound 48/80. The membrane action of zinc. *Agents Actions*, **4,** 320–323.

KEEN, C.L. & GERSHWIN, M.W. (1990). Zinc deficiency and immune function. *Annu. Rev. Nutr.*, **10,** 415–431.

KIREMIDJIAN-SCHUMACHER, L., STOTZKY, G., LIKKITE, V., SCHWARTZ, J. & DICKSTEIN, R.A. (1981). Influence of cadmium, lead and zinc on the ability of guinea-pig macrophages to interact with macrophage migration inhibitory factor. *Env. Res.*, **24,** 106–116.

KOIZUMI, S., OTAKI, N., SAEGUSA, J. & OTSUKA, F. (1993). Analysis of mammalian metallothionein isoforms by high-resolution SDS-gel electrophoresis. *Toxicol. Lett.*, **66,** 165–174.

KOJ, A.. (1985). Regulation of synthesis of acute phase proteins. In *The Acute Response to Injury and Infection*. ed. Gordon, A.J. & Koj, A. pp. 215–216. Amsterdam: Elsevier.

KOLLER, L.D. & ROAN, J.G. (1977). Effects of lead and cadmium on mouse peritoneal macrophages. *J. Reticuloend. Soc.*, **21,** 7–12.

KOROPATNICK, J., ANDREWS, G., DUERKSEN, J.D., VARSHNEY, U. & GEDAMU, L. (1983). Mouse hepatic metallothionein-1 gene cleavage by micrococcal nuclease is enhanced after induction by cadmium. *Nucleic Acids Res.*, **11,** 3255–3267.

KOROPATNICK, J. & LEIBBRANDT, M.E.I. (1995). Effects of metals on gene expression. In *Handbook of Experimental Pharmacology (Toxicology of Metals – Biochemical Aspects)*, Vol. 115. ed. Goyer, R.A. & Cherian, M.G. pp. 93–120. Heidelberg: Springer-Verlag.

KUBICKA-MURANY, M., BEHMER, O., UHRBERG, M., KLONOWSKI, H., BISTER, J. & BLEICHMANN, E. (1993). Murine systemic autoimmune disease induced by mercuric chloride ($HgCl_2$): Hg-specific helper T-cells react to antigen stored in macrophages. *Int. J. Immunopharmacol.*, **15,** 151–161.

LAWRENCE, D.A. & McCABE, JR., M.J. (1995). Immune modulation by toxic metals. In *Metal Toxicology*. ed. Goyer, R.A., Klaassen, C.D. & Waalkes, M.P. pp. 305–337. San Diego: Academic Press.

LAWRENCE, D.A. & SLAVIK, J. (1992). In vitro effects of metals on various reactivities of human peripheral blood and splenic mononuclear cells. *The Toxicologist*, **12,** 29.

LEIBBRANDT, M.E.I., KHOKHA, R. & KOROPATNICK, J. (1994). Antisense down-regulation of metallothionein in a human monocytic cell line alters anherence, invasion, and the respiratory burst. *Cell Growth Differ.*, **5,** 17–25.

LEIBBRANDT, M.E.I. & KOROPATNICK, J. (1994). Activation of human monocytes with lipopolysaccharide induces metallothionein expression and is diminished by zinc. *Toxicol. Appl. Pharmacol.*, **124,** 72–81.

LEIBBRANDT, M.E.I., KOROPATNICK, J., HARRIS, J.F. & CHERIAN, M.G. (1991). A radioimmunoassay to measure metallothionein in rodent and human tissues. *Biochem. Trace Element Res.*, **30,** 245–256.

LYNES, M.A., BORGHESI, L.A., YOUN, J. & OLSON, E.A. (1993). Immunomodulatory activities of extracellular metallothionein. I. Metallothionein effects on antibody production. *Toxicology*, **85,** 161–177.

MARCH, C.J., MOSLEY, B., LARSEN, A., CERRETTI, D.P., BRAEDT, G., PRICE, V., GILLIS, S., HENNEY, C.S., KRONHEIM, S.R., GRABSTEIN, K., CONLON, P.J., HOPP, T.P. & COSMAN, D. (1985). Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature*, **315,** 641–647.

MARONE, G., FINDLAY, S.R. & LICHTENSTEIN, M. (1981). Modulation of histamine release from human basophils in vitro by physiological concentrations of zinc. *J. Pharmacol. Exp. Ther.*, **217,** 292–298.

MONESTIER, M., LOSMAN, M.J., NOVICK, K.E. & ARIS, J.P. (1994). Molecular analysis of mercury-induced antibodies in H-2^2 mice. *J. Immunol.*, **152,** 667–675.

MOYNAHAN, E.J. (1981). Accrodermatitis enteropathica and the immunological role of zinc. *Immunodermatology*, **30,** 437–447.

MULLER, L. & MENZEL, H. (1990). Studies on the efficacy of lopoate and dihydrolipoate in the alteration of Cd^{2+} toxicity in isolated hepatocytes. *Biochim. Biophys. Acta*, **1052,** 386–391.

NAKAMURA, T., SHIRAISHI, N. & AONO, K. (1987). Effects of in vitro and in vivo supplementation with zinc on superoxide anion production in leukocytes. *Physiol. Chem. Phys. Med. NMR,* 19, 147–151.

NISHIZUKA, Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science*, **258,** 607–614.

O'HALLORAN, T.V. (1993). Transition metals in control of gene expression. *Science*, **261,** 715–725.

OLIVEIRA, D.B.G., FOSTER, G., SAVILL, J., SYME, P.D. & TAYLOR, A. (1987). Membranous nephropathy caused by mercury-containing skin lightening cream. *Postgrad. Med. J.*, **63,** 303–304.

PABST, M.J. & JOHNSTON, R.B. (1980). Increased production of superoxide anions by macrophages exposed in vitro to muramyl dipeptide or lipopolysaccharide. *J. Exp. Med.*, **151,** 101–114.

PELLETIER, L., PASQUIER, R., HIRSCH, F., SAPIN, C. & DRUET, P. (1985). In vivo self-reactivity of mononuclear cells to T cells and macrophages exposed to $HgCl_2$. *Eur. J. Immunol.*, **15,** 460–465.

PELLETIER, L., TOURNADE, H. & DRUET, P. (1990). Immunological mediated manifestations of metals. In *Immunotoxicity of Metals and Immunotoxicology*. ed. Dayan, A.D., Hertel, R.F., Heseltin, E., Kazantzis, G., Smith, E.M. & van der Venne, M.T. pp. 133–138. New York: Plenum.

PICK, E. & KEISARI, Y. (1980). A simple colorimetric method for the measurement of hydrogen peroxide by cells in culture. *J. Immunol. Methods*, **38,** 161–170.

REUTER, R., TESSARS, G., VOHR, H., GLEICHMANN, E. & LUHRMANN, A. (1989). Mercuric chloride induces autoantibodies against U3 small nuclear ribonucleoprotein in susceptible mice. *Proc. Natl. Acad. Sci. U.S.A.*, **86,** 237–241.

RUNGBY, J. & ERNST, E. (1992). Experimentally-induced lipid peroxidation after exposure to chromium, mercury or silver: interactions with carbon tetrachloride. *Pharmacol. Toxicol.*, **70,** 205–207.

SAIJOH, K., JUNO, T., SHUNTOH, H., TANAKA, C. & SUMINO, K. (1989). Molecular cloning of cDNA for rat brain metallothionein-II and regulation of its gene expression. *Pharmacol. Toxicol.*, **64,** 464–468.

SAMBROOK, J., FRITSCH, E.F. & MANIATIS, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor University Press.

SANCEAU, J., KAISHO, T., HIRANO, T. & WIETZERBIN, J. (1995). Triggering of the human interleukin-6 gene by interferon-γ and tumor necrosis factor-α in monocytic cells involves cooperation between interferon regulatory factor-1, NFκB, and Sp1 transcription factors. *J. Biol. Chem.*, **270,** 27920–27931.

SHENKER, B.J., MAYRO, J.S., ROONEY, C., VITALE, L. & SHAPIRO, I.M. (1993). Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. IV. Alterations in cellular glutathione content. *Immunopharmacol. Immunotoxicol.*, **15,** 273–290.

TAMAI, K.T., GRALLA, E.B., ELLERBY, L.M., VALENTINE, J.S. & THIELE, D.J. (1993). Yeast and mammalian metallothioneins functionally substitute for yeast copper-zinc superoxide dismutase. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 8013–8017.

THOMAS, P.T., RATAJCZAK, H.V., ARANYI, C., GIBBONS, R. & FENTERS, J.D. (1985). Evaluation of host resistance and immune function in cadmium-exposed mice. *Toxicol. Appl. Pharmacol.*, **80,** 446–456.

TUBBS, R.R., GORDON, D.O., GEPHARDT, N., MCMAHON, J.T., POHL, M.C., VIDT, D.G., BARENBURG, S.A. & VALENZUELS, R. (1982). Membranous glomerulonephritis associated with industrial mercury exposure. *Am. J. Clin. Pathol.*, **77,** 409–413.

VRUWINK, K.G., FLETCHER, M.P., KEEN, C.L., GOLUB, M.S., HENDRICKX, A.G. & GERSHWIN, M.E. (1991). Moderate zinc deficiency in rhesus monkeys. An intrinsic defect on neutrophil chemotaxis. *J. Immunol.*, **146,** 244–249.

WINCHURCH, R.A. (1988). Activation of thymocyte responses to IL-1 by zinc. *Clin. Immunol. Immunopathol.*, **47,** 174–180.

WIRTH, J.J., FRAKER, P.J. & KIERSZENBAUM, F. (1989). Zinc requirement for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. *Immunology*, **68,** 114–119.

ZALUPS, R.K. & LASH, L.H. (1994). Advances in understanding the renal transport and toxicity of mercury. *J. Toxicol. Environ. Health.*, **42,** 1–44.

ZENG, J., VALLEE, B.L. & KAGI, J.H.R. (1991). Zinc transfer from transcription factor IIIa to thionein clusters. *Proc. Natl. Acad. Sci. U.S.A.*, **88,** 9984–9988.

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