Metallothionein mRNA and protein induction by cadmium in peripheral-blood leucocytes

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To help to evaluate the role of metallothionein (MT) in peripheral-blood leucocytes, we examined MT protein and mRNA levels in these cells before and after exposure to CdCl₂ in culture. Protein was assayed by ¹⁰⁹Cd²⁺ binding, and RNA by dot-blot hybridization. MT was induced in both lymphocytes and adherent monocytes about 10-fold with a 12 h exposure to 10 μM-CdCl₂, but absolute levels were 3-fold higher in monocytes: 57 × 10⁵ (+Cd²⁺) versus 6 × 10⁵ (–Cd²⁺) molecules/cell for monocytes; 18 × 10⁵ (+Cd²⁺) versus 2 × 10⁵ (–Cd²⁺) for lymphocytes. Polymorphonuclear cells expressed relatively little MT (0.6 × 10⁵ molecules/cell), and this did not change with phorbol ester stimulation or exposure to Cd²⁺, arguing against a direct protective role for MT in activated neutrophils. MT mRNA levels corresponded qualitatively to expression of protein in these cells. Our data provide quantitative comparisons of leucocyte MT expression and regulation in the human population. Variation in both basal and induced MT mRNA levels reflects environmental or experimental (intra-individual) and possibly genetic (inter-individual) differences.

INTRODUCTION

Metallothioneins (MT) are small cysteine-rich proteins which bind group IB (copper, silver, gold) and IIB (zinc, cadmium, mercury) metal ions with high affinity (see Hamer, 1986). In almost all vertebrate cells and tissues studied, MT protein levels are induced by exposure to these metal ions. However, other agents, such as glucocorticoids (Hager & Palmiter, 1981), interferon (Friedman *et al.*, 1984), interleukin-1 (Durnam *et al.*, 1984; Karin *et al.*, 1985) and activators of protein kinase C (Imbra & Karin, 1987), also induce MT expression in some cell types. Induction is known to occur primarily at the level of transcription through factors which act on proximal promoter elements, but the mechanism of regulation is not well understood.

The role of MT in normal physiology is not known. It is not essential for cell growth, since cell lines devoid of MT are capable of normal growth and proliferation. However, these cells are sensitive to elevated levels of inducing metal ions, whereas cells which over-express MT are resistant. These data suggest a role for MT in metal detoxification (Hamer, 1986; MacArthur & Lieberman, 1987). However, MTs constitute a multigene family, and the complex tissue distribution, developmental regulation of MT isoforms, and the ability of MT to deliver essential Zn²⁺ and Cu²⁺ ions to metalloenzymes, suggest that MT may also be involved in aspects of normal growth and development in multicellular organisms. In addition, it has been suggested that MT may protect cells against damage caused by free radicals produced, for example, by radiation or the phagocyte superoxide burst (Thornalley & Vasak, 1985; Thomas *et al.*, 1986; Peavy & Fairchild, 1987; Matsubara *et al.*, 1987). Thus the inducibility of MT is of considerable interest, since this protein may be a key protective agent during a variety of stress conditions.

Peripheral-blood leucocytes provide a convenient tissue in which to study MT expression, since they synthesize MT and respond to concentrations of cadmium comparable with that found in cases of occupational human exposure (Hildebrandt & Cram, 1979). Work by Peavy & Fairchild (1987) has shown that cadmium induces MT in mononuclear cells (lymphocytes and monocytes), but not in polymorphonuclear cells, and that none of these cells respond directly to bacterial lipopolysaccharide, interleukin-1, or leucocyte endogenous mediator. Thus the protective role of MT in leucocytes exposed to inflammatory agents apparently occurs through indirect MT induction, presumably by elevated levels of endogenous corticosteroids. To evaluate further the role of MT in peripheral-blood cells, we studied variation in basal and Cd²⁺-induced MT protein and mRNA in lymphocytes, monocytes and polymorphonuclear cells in the human population.

EXPERIMENTAL

Cells

Peripheral blood mononuclear leucocytes (about 85 % lymphocytes, 15 % monocytes) were isolated from fresh heparinized blood removed by venipuncture from healthy non-smoking donors (aged 20–40 years) by centrifugation over Ficoll–Hypaque [5.7 % (w/v) Ficoll 400, 9 % (w/v) sodium diatrizoate] as described by Boyum (1968). Mononuclear leucocytes were carefully removed, washed in phosphate-buffered saline (0.88 mM-KH₂PO₄, 6.4 mM-Na₂HPO₄, 137 mM-NaCl, 2.7 mM-KCl, pH 7.4) and resuspended in supplemented RPMI 1640 [62.5 units of penicillin/ml, 62.5 μg of streptomycin/ml, 0.16 μg of fungizone/ml, 1.25 mM-L-glutamine, 5 mM-Hepes, and 20 % (v/v) fetal bovine serum, final pH 7.5–7.6]. Polymorphonuclear cells were isolated from the Ficoll–

Abbreviations used: MT, metallothionein; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

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Hypaque gradient and purified by lysing contaminating red blood cells as described by Boyum (1968).

Cadmium induction

Cells were assayed for viability by Trypan Blue exclusion, plated at 2×10^6 cells/ml in Petri dishes with or without CdCl_2 at indicated concentrations and incubated at 37°C and 100% humidity, under 5% CO_2 , for indicated times. Standard conditions were $10 \mu\text{M}$ - CdCl_2 for 6 h (RNA studies) or 12 h (protein studies). Suspension and adherent cells (liberated from the Petri dish with a rubber policeman) were transferred to 15 ml tubes, centrifuged (1400 g , 10 min), and treated as indicated below for determination of MT protein or RNA. In other experiments, adherent and non-adherent cells were separated after 6 h in culture before the 6 h treatment with Cd^{2+} . The former protocol was adopted for routine use, since it gave essentially identical results and required less manipulation of the cells. In no case was significant MT induction observed in response to culturing cells in regular growth medium.

MT protein determination

Cell pellets were resuspended in 600 μl of 10 mM-Tris/HCl, pH 8.0, and lysed by sonication (40 s, Bronson Sonicator). The lysate was centrifuged (12000 g , 15 min) and the supernatant heated (70°C), cooled to room temperature, and re-spun (12000 g , 15 min). The supernatant (400 μl) was then added to 100 μl of $^{109}\text{CdCl}_2$ [$1 \mu\text{Ci}/10 \mu\text{g}$ per 100 μl in 9% (w/v) NaCl], equilibrated for 15 min at room temperature, and cleared of excess $^{109}\text{Cd}^{2+}$ with three haemolysate precipitations (Onosaka & Cherian, 1982) by using fresh haemolysate as described by Nolan & Shaikh (1986). Samples of the final supernatant were removed for determination of radioactivity measured on a γ -radiation counter. The amount of MT protein was calculated, after background subtraction, assuming specific binding of 7 mol of Cd^{2+} /mol of MT. This technique, when used in conjunction with column chromatography and known MT standards periodically to confirm specific binding and accuracy, was found to provide a rapid and highly reproducible measure of MT (see also Dieter *et al.*, 1986).

MT mRNA determination

After isolation of specific populations of peripheral-blood leucocytes, cell pellets were immediately lysed and RNA was purified by the guanidinium isothiocyanate method (Glisin *et al.*, 1974) as described previously (Harley, 1987). To determine concentration and purity, samples of each RNA preparation were quantified with a fluorimeter at pH 8.1 and 11.8 with and without RNAase treatment (Morgan *et al.*, 1979; Harley, 1987). Samples containing more than 6% DNA were discarded or re-purified. Samples were denatured and spotted on to nitrocellulose in duplicate or quadruplicate for dot-blot analysis using one or two ^{32}P -labelled MT-specific probes [synthetic oligonucleotides complementary to the coding region of both human MT-I and MT-II (Hanke *et al.*, 1988)] and a normalization probe [^{32}P -labelled (dT)₁₈] (Harley, 1987). Specificity of the MT probes was shown with plasmids containing MT inserts (see Fig. 7 and Hanke *et al.*, 1988). MT probes were hybridized at 48°C for 18–20 h in hybridization buffer [$5 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M-NaCl}/0.015 \text{ M-sodium citrate}$, pH 7), $5 \times \text{Denhardt's solution}$ (0.1% Ficoll, 0.1% polyvinylpyrrolidone,

0.1% bovine serum albumin), 0.01 M- Na_2HPO_4 , 1 mM- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$] containing 6 pmol of labelled probe (Bell *et al.*, 1987). Filters were washed for 4×5 min in $1 \times \text{SSC}$ at 48°C . Oligo(dT)-probed filters were hybridized and washed as described previously (Harley, 1987). Autoradiograms, exposed within the linear range of film response, were scanned with a densitometer. Readings were normalized by using standard curves generated by applying known amounts of radioactivity to the nitrocellulose. Note that the dot-blot assay measures total cellular MT RNA, which includes both hnRNA and mRNA.

Cytotoxicity of CdCl_2

Cells were incubated under standard conditions for 24 h in a 24-well plate with indicated concentrations of CdCl_2 . Incubation continued for 4 h after adding $1 \mu\text{Ci}$ of [^3H]phenylalanine (50 Ci/mmol). In some experiments, cells were preincubated with or without $10 \mu\text{M}$ - CdCl_2 for 12 h. At the end of the [^3H]phenylalanine incubation, cells were harvested and assayed for viability by Trypan Blue exclusion and for incorporation of radioactivity into trichloroacetic acid-insoluble material (Harley *et al.*, 1980).

Cadmium and zinc determinations

Cadmium and zinc were measured in serum and tissue-culture medium (containing 20% serum) by electrothermal atomic absorption spectrometry after acid deproteinization (Stoepler & Brandt, 1980).

Fig. 1. Gel-filtration chromatography of $^{109}\text{Cd}^{2+}$ radioactivity from cell

Cell lysates from peripheral-blood mononuclear cells cultured for 12 h without (Δ) or with ($\circ$) $10 \mu\text{M}$ - CdCl_2 were prepared as described in the Experimental section and applied to a Sephadex G-75 column (41 cm $\times$ 1 cm). The positions at which lysozyme (L) (M_r 14000) and ovalbumin (O) (M_r 43000) were eluted from the column with Tris buffer (10 mM-Tris/15 mM-NaCl, pH 8.0) are shown by arrows. A representative profile from three independent experiments is shown.

RESULTS AND DISCUSSION

MT protein

Levels of MT protein in peripheral-blood leucocytes were determined by the cadmium-saturation assay, which measures the amount of $^{109}\text{Cd}^{2+}$ specifically bound to heat-stable protein (Onosaka & Cherian, 1982; Nolan & Shaikh, 1986). In our experience, high- M_r proteins or residual free $^{109}\text{Cd}^{2+}$ did not contribute significantly to the measured amounts of bound $^{109}\text{Cd}^{2+}$ in either induced or uninduced cells (Fig. 1). The minor shoulder on the MT peak represents $^{109}\text{Cd}^{2+}$ bound to a species which co-eluted with lysozyme (M_r 14000) and likely represents dimers of MT. Since analyses of column-fractionated material varied only slightly from analysis of total lysates, routine calculations used total $^{109}\text{Cd}^{2+}$ bound to heat-stable protein.

The cytotoxicity of Cd^{2+} in cultured mononuclear leucocytes was measured by cell viability and protein synthesis after 24 h exposure (Figs. 2a and 2b). Gross cytotoxic effects, evident by inability to exclude Trypan Blue and decreased incorporation of $[^3\text{H}]$ phenylalanine, were observed only with Cd^{2+} concentrations above $100\ \mu\text{M}$. In whole blood, even higher concentrations of added Cd^{2+} are required to evoke maximal leucocyte response, presumably owing to the buffering capacity of red blood cells (C. B. Harley, unpublished work). Pre-exposure of cultured mononucleated cells to sub-cytotoxic ($10\ \mu\text{M}$) levels of Cd^{2+} did not prevent Cd^{2+} -induced decreases in cell viability or rate of protein synthesis (Fig. 2a). Our results are in contrast with others which show that pre-exposure to Cd^{2+} protects macrophages from cytotoxic effects of lipopolysaccharide (Patierno *et al.*, 1983), kidney cells from Cd^{2+} (Jin *et al.*, 1987), and mice from irradiation (Matsubara *et al.*, 1987). This discrepancy could reflect tissue or assay differences, or a specific dose- and time-dependence for the protective effect.

Fig. 2. Cytotoxic effects of Cd^{2+} ions on cultured peripheral-blood mononuclear cells

(a) Cells were incubated with the indicated concentrations of CdCl_2 for 24 h and assayed for cell viability by Trypan Blue exclusion without (Δ) or with ($\circ$) prior incubation for 12 h with $10\ \mu\text{M}$ CdCl_2 . (b) Incorporation of $[^3\text{H}]$ phenylalanine into acid-insoluble material after incubation with indicated concentrations of CdCl_2 was determined as described in the Experimental section. Bars represent s.d. Data from one of two similar experiments are shown.

Fig. 3. Dose and time responses of MT protein levels in peripheral-blood mononuclear cells cultured with CdCl_2

Cells were incubated for 12 h with the indicated concentrations of CdCl_2 (a), or with (Δ) 0 or ($\circ$) $10\ \mu\text{M}$ CdCl_2 for the indicated times (b) before determination of MT levels as described in the Experimental section. Cell number did not change significantly during this period. Data from one of four similar experiments are shown.

Table 1. Variation in Cd²⁺ induction of MT in mononuclear cells

Mononuclear cells were isolated and cultured with or without CdCl₂ for 12 h before MT determination as described in the text. Values shown are means from at least two determinations for each donor.

Donor	10 ⁻⁵ × MT content (molecules/cell)		Induction ratio
	Basal	Induced	
D4	2.6	26.3	10.1
D5	3.9	37.0	9.5
D17	1.5	19.8	13.2
D18	2.2	16.5	7.5
D19	2.9	20.2	7.3
D20	3.9	19.6	5.1
D21	2.0	15.3	7.5
Mean	2.7 ± 0.9	22.1 ± 7.4	8.6 ± 2.6

The dose-response of cultured mononuclear leucocytes for MT levels after 12 h exposure to Cd²⁺ is shown in Fig. 3(a). MT levels increased with Cd²⁺ concentration up to 100 µM. At higher concentrations, MT levels decreased dramatically, presumably owing to cytotoxic effects (Fig. 2). Since no plateau in induction was observed at sub-cytotoxic levels of Cd²⁺, we chose 10 µM-Cd²⁺ as the standard concentration in these experiments. This concentration is not acutely cytotoxic (Fig. 2), and is similar to Cd²⁺ concentrations used in other studies (Karin *et al.*, 1984; Schmidt & Hamer, 1986; Sone *et al.*, 1987). MT levels increase dramatically with time in culture when cells were exposed to 10 µM-Cd²⁺ (Fig. 3b). To avoid possible variables inherent in longer-term culture, we arbitrarily chose 12 h as the standard incubation time for measuring MT protein levels.

Variation in MT levels in peripheral-blood mononuclear leucocytes before and after exposure to 10 µM-Cd²⁺ for 12 h in culture for normal adult donors is shown in Table 1. The induction ratio varied from 5- to 13-fold, which reflected about 2-fold variation in both basal and induced levels of MT. It is unlikely that variation in basal MT content reflects variation in endogenous Cd²⁺, since blood Cd²⁺ is typically < 0.1 µM and rarely exceeds 0.5 µM (Roels *et al.*, 1983; E. Nieboer, unpublished work). However, low levels of MT induction in these cells by environmental factors cannot be excluded, and may in fact be diagnostic of metal exposure. Other factors, such as genetic differences in metal uptake or MT expression, or differences in copper, zinc or glucocorticoid levels, possibly account for the observed variation between donors in both basal and induced levels of MT. To eliminate variation in metal or hormonal stimuli from culture medium used for cells *in vitro*, a single lot of fetal-bovine serum was used throughout these studies. The measured concentrations of Cd²⁺ and Zn²⁺ in the final culture medium was less than 0.2 µM and 2.0 µM respectively, and in fact no component of the medium, including possible serum MT, caused significant induction of cellular MT (Fig. 3b). Variation between experiments for a given individual could reflect experimental error and/or the specific immune and endocrine status of the individual at the time of the blood collection.

Fig. 4. Cell specificity of MT expression in peripheral-blood leucocytes

Leucocytes were fractionated into total mononuclear cells (MNL), non-adherent mononuclear cells (lymphocytes, L), adherent mononuclear cells (monocytes, M) and polymorphonuclear cells (PMN) as described in the Experimental section. MT levels were determined after 12 h incubation without (■) or with (□) 10 µM-CdCl₂. S.D. values from determinations on at least three different donors are shown above the histograms. The induction ratios (mean induced to mean uninduced MT levels) for each cell population are shown within the white histograms.

Table 2. MT content of polymorphonuclear cells stimulated with TPA

Polymorphonuclear cells were isolated and incubated with TPA as indicated before determining MT content as described in the text. Values shown are from a representative experiment.

Time (h)	[TPA] (nM)...	10 ⁻⁵ × MT content (molecules/cell)			
		0	1	10	100
0		1.41	—	—	—
0.08		—	0.79	2.50	0.95
6		0.76	0.85	0.66	0.66
12		1.10	1.18	0.66	0.73

The donor-specific induced levels of MT expression vary less between experiments, and are thus expected to be more indicative of the inherent ability to respond to Cd²⁺ induction.

To compare MT levels in both major types of mononuclear leucocytes (lymphocytes and monocytes) and in polymorphonuclear cells (mainly neutrophils), peripheral-blood leucocytes were further fractionated. Monocytes contained about 3 times more MT than did lymphocytes in both basal and Cd²⁺-induced conditions (Fig. 4). Total mononuclear-leucocyte MT levels were in agreement with the weighted contribution of lymphocytes

Fig. 5. Dose and time response of MT mRNA levels in peripheral-blood mononuclear cells cultured with CdCl₂.

Cells were incubated for 6 h with the indicated concentrations of CdCl₂ (a) or with (△) 0 or (○) 10 μM-CdCl₂ for the indicated times (b) before determination of MT mRNA levels. Symbols □, △, ○ in (a) denote separate donors. Inserts show dot-blot hybridization (a) for donor D16 at the indicated concentrations of CdCl₂, and (b) for donor D4 at the indicated times (h), with (+) or without (-) CdCl₂. In (a), Cd²⁺-minus controls were assayed without (O_B) or with (O_A) a 6 h incubation in culture. Data from one of two similar experiments are shown.

Fig. 6. Frequency distribution of normal adult donors with respect to MR mRNA induction ratio

The inset shows an autoradiogram from a typical experiment in which RNA from peripheral-blood mononuclear cells cultured without (-) or with (+) 10 μM-CdCl₂ for 6 h was spotted on to nitrocellulose and hybridized with a complementary MT oligonucleotide. The histogram shows the distribution of mean induction ratios of 39 normal adult donors, calculated from analysis of all such RNA dot-blot. The S.D. for the basal MT mRNA level in the population shown in the inset was 30% of the mean, whereas that for the Cd²⁺-induced levels was 40% of the mean.

and monocytes (85% and 15% of the cell population respectively). Polymorphonuclear cells, in contrast, contained very little MT either with or without induction *in vitro* with CdCl₂. These results are in accordance with the report by Peavy & Fairchild (1987), in which polymorphonuclear cells were found to have low levels of MT that were not inducible with lipopolysaccharide or Cd²⁺, whereas MT induction by Cd²⁺ in monocytes and (to a lesser extent) lymphocytes was significant.

To determine whether MT increases in activated neutrophils, isolated polymorphonuclear cells were incubated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) at concentrations known to activate protein kinase C and to induce the superoxide burst (Table 2). However, TPA did not induce a consistent increase in MT levels at any of the concentrations or times tested. We also found no evidence for TPA induction of MT in the cultured human promyelocytic leukaemia-cell line HL-60 in either the undifferentiated or neutrophil-like differentiated state (Hanke *et al.*, 1988). These data argue against a critical role for protein kinase C activation in MT induction (Imbra & Karin, 1987) or a protective role for MT in the oxidative environment of the activated neutrophil.

MT mRNA

Levels of MT mRNA were determined in parallel with most experiments on MT protein by semi-quantitative dot-blot analysis by using synthetic oligonucleotides complementary to MT mRNA (Hanke *et al.*, 1988). MT signals were normalized by using labelled (dT)₁₈, which specifically hybridizes to poly(A), thus generating a total mRNA signal which was not dependent on short-term exposure to Cd²⁺ (Harley, 1987; Hanke *et al.*, 1988).

Fig. 7. Autoradiograms of dot-blots of peripheral-blood mononuclear-cell total RNA cultured without (–) or with (+) $10 \mu\text{M}$ - CdCl_2 for 12 h

The blot was probed with oligonucleotide MTAP (Hanke *et al.*, 1988) (left panel) or $(\text{dT})_{18}$ (right panel). Dots at the bottom of the left panel represent plasmid DNA with a human MT-IIA genomic DNA insert (pAS6). The number of denatured plasmid molecules applied to the filter is indicated above each of these dots.

Table 3. Individual variation in MT mRNA levels

Densitometric scans of RNA dot-blots shown in Fig. 7 were quantified for three donors in three separate experiments as described in the Experimental section. Values represent means $\pm$ S.D., in arbitrary units.

	D19	D20	D9
–Cd	0.12 ± 0.03	0.12 ± 0.02	0.14 ± 0.02
+Cd	0.85 ± 0.20	1.25 ± 0.25	1.48 ± 0.20
Ratio	7.19 ± 0.35	10.0 ± 1.14	10.6 ± 0.93

Northern-blot analyses gave similar results (not shown), but were less reproducible than dot-blots.

The dose- and time-dependence of MT mRNA levels on exposure to CdCl_2 in cultured mononucleated leucocytes is shown in Fig. 5. Interestingly, very high doses of Cd^{2+} (greater than $100 \mu\text{M}$), which are clearly cytotoxic at 24 h (Fig. 2) and yield very little MT protein in cells after 12 h (Fig. 3), gave maximal MT mRNA levels at 6 h (Fig. 5a). It is not known whether the mechanism for this high-level induction of MT mRNA is different from that at lower Cd^{2+} concentrations. Koropatnick *et al.* (1985) reported MT gene amplification in mouse liver after acute exposure to high levels of Cd^{2+} *in vivo*. However, we observed no evidence of MT-gene amplification on Southern-blot analysis of mononuclear leucocytes treated with $320 \mu\text{M}$ - Cd^{2+} (results not shown). The standard concentration ($10 \mu\text{M}$ - Cd^{2+}) used in MT protein determinations gave a near-maximal response in the sub-cytotoxic range of Cd^{2+} concentrations ($< 100 \mu\text{M}$), and thus was used in subsequent studies of MT mRNA

levels. MT mRNA levels of cultured mononuclear cells [normalized to total polyadenylated RNA] reached a peak after 6–12 h when incubated with $10 \mu\text{M}$ - CdCl_2 (Fig. 5b). The continued accumulation of MT protein (Fig. 3b) after the point at which MT mRNA reaches a plateau (Fig. 5b) presumably reflects the longer half-life of Cd-MT protein ($\gg 1$ day) compared with MT mRNA (< 1 day) (see Durnam & Palmiter, 1981; Monia *et al.*, 1986).

Individual variation in MT mRNA levels in peripheral-blood mononuclear leucocytes before and after exposure to $10 \mu\text{M}$ - Cd^{2+} for 6 h in culture is shown in Fig. 6 for 39 normal adult donors aged 20–40 years. Both basal and induced MT mRNA levels varied markedly from individual to individual, giving rise to a large range in induction ratio [4.3–38-fold; mean = 17 ± 8 (S.D.)]. To assess the contribution of experimental error to the between-donor variation seen in basal and induced levels of MT mRNA, three different individuals were analysed over a short period of time (3 weeks) in three separate experiments. Fig. 7 shows the results of these experiments, as well as those from other donors. Donors D20 and D9 both had significantly elevated MT induction under these conditions compared with donor D19 ($P < 0.05$, two-tailed Student's *t* test) (Table 3). This difference in induction ratio in this set of experiments resulted from an elevation in induced levels of RNA, rather than changes in basal MT mRNA expression.

Conclusions

Our results show that levels of MT protein and mRNA in peripheral-blood leucocytes are cell- and donor-specific and very sensitive to the dose and duration of cadmium exposure. For example, at high concentrations of Cd^{2+} ($> 100 \mu\text{M}$), cytotoxicity led to low measured values of

MT protein at 12 h, despite high levels of MT mRNA at 6 h. At lower concentrations of Cd^{2+} , MT protein accumulated over an extended period of time (> 60 h), whereas MT mRNA reached a peak at about 12 h. These data are partially explained by the higher rate of turnover of MT mRNA compared to MT-Cd protein (Durnam & Palmiter, 1981; Monia *et al.*, 1986). Adherent monocytes had higher basal and Cd^{2+} -induced levels of MT compared with lymphocytes, whereas polymorphonuclear cells expressed relatively little MT, and this was not increased after exposure to Cd^{2+} . Variation in basal and induced levels of MT expression appear to reflect both environmental and experimental factors and possibly genetic differences. Although the origin of the latter variation is unknown, it could be relevant to differential responses to occupational exposure to cadmium.

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