Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology 222 (2006) 37–45
TOXICOLOGY
www.elsevier.com/locate/toxicol

The in vitro immune modulation by cadmium depends on the way of cell activation

Nasr Y.A. Hemdan [a,b,c,*], Frank Emmrich [a], Ulrich Sack [a], Gunnar Wichmann [b], Jörg Lehmann [d,1], Khadiga Adham [c], Irina Lehmann [b]

[a] *Institute of Clinical Immunology and Transfusion Medicine, University of Leipzig, Germany*
[b] *Department of Environmental Immunology, UFZ-Centre for Environmental Research Leipzig-Halle, Germany*
[c] *Department of Zoology, Faculty of Science, University of Alexandria, Egypt*
[d] *Institute of Immunology, Faculty of Veterinary Medicine, University of Leipzig, Germany*

Received 15 December 2005; received in revised form 16 January 2006; accepted 17 January 2006
Available online 28 February 2006

Abstract

Among environmental contaminants known for their toxicity and worldwide distribution, heavy metals are of primary concern. Although the toxicology of cadmium (Cd) has been extensively studied, little information is available on the immunomodulation driven by exposure to low doses of Cd. We aimed to evaluate the immunomodulatory effects elicited by short-term exposure of human immunocompetent cells to low biologically relevant doses of Cd in two activation models. Human peripheral blood mononuclear cells, activated either by bacterial antigens (heat-killed *Salmonella* Enteritidis) or monoclonal antibodies (mAb: anti-CD3/anti-CD28/anti-CD40), were exposed to Cd acetate for 24 h. Cell vitality was determined by MTT assay, cytokine release by ELISA, and cytokine gene expression by real-time RT-PCR. The results demonstrated that, in addition to the known toxic effects of Cd, doses from 0.013 to 13.3 μM exert differential effects on cytokine production. In the case of mAb-activation, secretion of interleukin (IL)-1β, tumour necrosis factor (TNF)-α and interferon (IFN)-γ was greatly inhibited at low Cd doses compared to production of IL-4 and IL-10. This indicates a type-2-biased immune response. Under stimulation by bacterial antigens, release of IL-10 was highly suppressed compared to that of IFN-γ and TNF-α; IL-4 was undetectable. These results imply that low Cd doses exert immunomodulatory effects and the direction of this modulation depends on the pathway to cell activation. Overall, Cd polarizes the immune response toward type-2 in cells stimulated via T cell receptors. However, a polarized type-1 response induced by bacterial antigens could not be overwhelmed by the effects of Cd.

Keywords: Cadmium acetate; Cell activation; T_H cytokine; Immune modulation; Type-1/2 immune response

1. Introduction

The influences of the heavy metals on human health have been debated for several decades. Through its various industrial uses, cadmium (Cd) has become an ever increasing environmental contaminant which is known to enter cells via Ca^{2+} channels of cellular membrane of many cells and accumulate intracellularly due to its binding to cytoplasmic and nuclear material (Beyersmann

* Corresponding author at: Institute of Clinical Immunology and Transfusion Medicine, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany. Tel.: +49 341 97 25821; fax: +49 341 97 25828.
E-mail address: hemdan.nasr@medizin.uni-leipzig.de (N.Y.A. Hemdan).
[1] Present address: Fraunhofer Institute for Cell Therapy and Immunology, Biocity, Leipzig, Germany.

0300-483X/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2006.01.026

and Hechtenberg, 1997; Satoh et al., 2003), constituting a potential threat to human and animal health. The primary route of Cd exposure is contaminated water or food supplies (Leffel et al., 2003; Satarug and Moore, 2004), smoking (Satarug and Moore, 2004), mother's milk (Pillet et al., 2005), through work in battery factories (Sahmoun et al., 2005), or through fertilizers or indiscriminate use of pesticide (Weggler et al., 2004).

Cadmium is toxic to humans and animals and excessive exposure to it results in diseases and occasionally death (Othumpangat et al., 2005). It is a well-known carcinogen (Satoh et al., 2002; Banerjee and Flores-Rozas, 2005) and a teratogenic agent (Hovland et al., 2000). It is also known to cause sterility (Bench et al., 1999), renal dysfunction (Cai et al., 2001), hepatic toxicity (Horiguchi et al., 2000), pancreatic activity changes (Shimada et al., 2000). Genotoxicity and apoptotic effects of Cd have been also reported in a number of cell types (Fahmy and Aly, 2000; Kim et al., 2005; Mondal et al., 2005).

Although immunomodulatory activities have been investigated in several in vivo and in vitro model systems, conflicting results have emerged (reviewed Satoh et al., 2003). For example, some publications have reported only insignificant in vivo immunosuppressive effects in mice (Borgman et al., 1986; Schulte et al., 1994; Chowdhury et al., 1987; Chopra et al., 1984a), in male rhesus monkeys orally exposed to 5 mg Cd/kg body weight for a period of 4 months (Chopra et al., 1985) and in man (Borella and Giardino, 1991) at doses in the range of 50–500 nM in vitro, where only a dose-independent mitogenic activity in unstimulated cells resulted and immunoglobulin secretion was not significantly affected in PWM-activated cells. A number of other studies have documented that Cd is not only toxic to living organisms but may modulate immune responses (Krocova et al., 2000; Leffel et al., 2003).

Exposure to Cd at sub-toxic levels has been documented to cause impairment of immunosurveillance, which controls expression and inactivation of viruses and bacteria. Significant depression in the phagocytic activity (Loose et al., 1978) as well as suppression of humoral and cell-mediated immune responses (Dan et al., 2000) were also reported. It was found that susceptibility to bacteria in Cd-exposed mice was due to a defect of macrophage recruitment to sites of infection (Simonet et al., 1984). Thus, it has been suggested that Cd increase susceptibility of affected individuals to bacterial and viral infections (Shen et al., 2001; Simonyte et al., 2003).

The available data concerning the effects of Cd on human cytokine responses, in particular T cell-derived cytokines, are fragmentary and contradictory. For example, Daniels et al. (1987) reported a significant depression of NK cell activity but no changes in IFN-γ production by Cd treatment. A significant enhancement in the IFN-γ production, however, was reported by Yucesoy et al. (1997) and by Krocova et al. (2000). Furthermore, Marth et al. (2000) stated that low Cd doses were able to stimulate the proinflammatory cytokines IL-1β, TNF-α and IL-6, whereas IFN-γ was only reactive at higher concentrations. Because of these inconstancies, the present study was conducted to investigate the effects of biologically-relevant low Cd doses on some immunoregulatory cytokine profiles using two ways of cell activation to complete and clarify the already established information.

2. Methods

2.1. Cell culture

Peripheral blood mononuclear cells (PBMC) were separated from buffy coats of healthy donors (14 samples) through density gradient centrifugation (Ficoll®; Amersham Biosciences, Freiburg, Germany) and suspended in HybridoMed DIF 1000 (Biochrom, Berlin, Germany). Cells in density of 1×10^6/ml were cultured in 48-well flat-bottom microtiter plates (Greiner Bio-one, Nürtingen, Germany) and stimulated with either monoclonal antibodies (mAbs) anti-CD3 (OKT3, mouse IgG1; Ortho Biotech, Bridgewater, NJ, USA), anti-CD28 (clone CD28.2, mouse IgG1; Beckman-Coulter, Krefeld, Germany) and anti-CD40 (clone B-B20, Trinova Biochem, Gießen, Germany), each at a final concentration of 100 ng/ml, or bacterial antigens (hk*S*E — heat-killed *Salmonella* Enteritidis; 1.25×10^5 cfu/ml).

2.2. Application of cadmium

A stock solution of Cd acetate (Sigma, Steinheim, Germany) was prepared in deionised water. Immediately before application, 10 serial concentrations from 0.013 to 444.8 μM Cd were made using the culture medium. Control samples were established, where cells received only either mAbs or hk*S*E.

2.3. MTT assay

The method was first developed by Mosmann (1983) and described by Hemdan et al. (2005). Following 24-h exposure to Cd and harvesting culture supernatant, remaining cells received 25 μl/well MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 5 g/L PBS; Sigma) and were incubated in the dark at 37 °C. After 4 h, 300 μl/well stop solution (10%, w/v sodium dodecylsulfate in 50%, v/v *N,N*′-dimethylformamide, SERVA, Heidelberg, Germany)

were added. The optical densities were measured after 24 h using an automatic plate reader (Spectra Image; Tecan, Crailsheim, Germany) at a wavelength of 570 nm and at 450 nm reference.

2.4. Cytokine release

The cytokines IL-1β, IL-10, IL-4, IFN-γ and TNF-α in culture supernatants were determined using commercially available ELISA Kits (OptEIA™ Kits; BD Biosciences, Heidelberg, Germany) and according to the manufacturer's instructions. The lower detection limits of these kits are 4 pg/ml. The optical density was measured by using an automatic plate reader (Spectra Image; Tecan) at 450 and at 620 nm for reference. Results were expressed as percentages of control.

2.5. Real-time RT-PCR

For RNA extraction, the E.Z.N.A. total RNA Kit (PeqLab Biotechnologie, Erlangen, Germany) was used following the manufacturer's instructions. The total RNA samples were kept at −20 °C for 1 day and then transcribed into cDNA immediately after thawing. The transcription into cDNA was done by AMV reverse transcriptase (Promega, Mannheim, Germany) at 42 °C for 60 min using the primer dNTP set and oligo-dT (5′-pd(T)$_{12–18}$-3′) (Amersham Biosciences). The resulting cDNA samples were kept at −20 °C. Real-time RT-PCR using the LightCycler FastStart DNA Master SYBR Green I reagent Kit (Roche Diagnostics, Mannheim, Germany) was performed for the housekeeping gene GAPDH, and the cytokine genes IFN-γ and IL-4 from the same RNA samples. The reaction was run by a LightCycler (Roche Diagnostics).

Following the manufacturer's instructions, 2 μl of the cDNA were amplified in 20 μl reaction mixture that contained $MgCl_2$ (4 mM for GAPDH, 5 mM for IL-4, and 3 mM for IFN-γ), cDNA Master, and 0.5 μM of both sense and antisense primers. The sequences of sense and antisense primers used were: (i) GAPDH: 5′-GTCAGTGGTGGACCTGACCT and 5′-AGGGGAGATTCAGTGTGGTG; (ii) IL-4: 5′-AGAAGACTCTGTGCACCGAGTTGA and 5′-CTCTCATGATCGTCTTTAGCCTTT; (iii) IFN-γ: 5′-TTCAGCTCTGCATCGTTTTG, and 5′-TCAGCCATCACTTGGATGAG, respectively.

Real-time RT-PCR reactions were performed using 40 cycles and started with a 10-min denaturation step at 95 °C. Different amplification protocols were used for each gene (GAPDH: 95 °C/0 s, 64 °C/5 s, 72 °C/17 s; IFN-γ: 95 °C/0 s, 64 °C/5 s, 72 °C/16 s; IL-4: 95 °C/1 s, 68 °C/10 s, 72 °C/16 s with lowering the annealing temperature of 0.5 °C per cycle until reaching a final annealing temperature of 58 °C at cycle 17). External standards (GenExpress, Berlin, Germany) were used for quantification. Specificity of the PCR product was controlled by the melting peaks. The results were corrected by calibration with GAPDH gene expression.

2.6. Statistical methods

All experiments were carried out in triplicates. Data analysis was performed using the STATISTICA 5.1 software (Statsoft, Hamburg, Germany). Differences between control and Cd-treated samples were tested using Wilcoxon test with an entry criterium of 0.05.

3. Results

Following 24-h exposure to Cd, cell vitality was significantly reduced compared to control groups in a dose-dependent relationship and independent of the activation method used (Fig. 1).

As expected, our results demonstrate that Cd doses which exerted cytotoxic effects (see Fig. 1) also decreased the mAbs- and hk*S*E-induced secretion

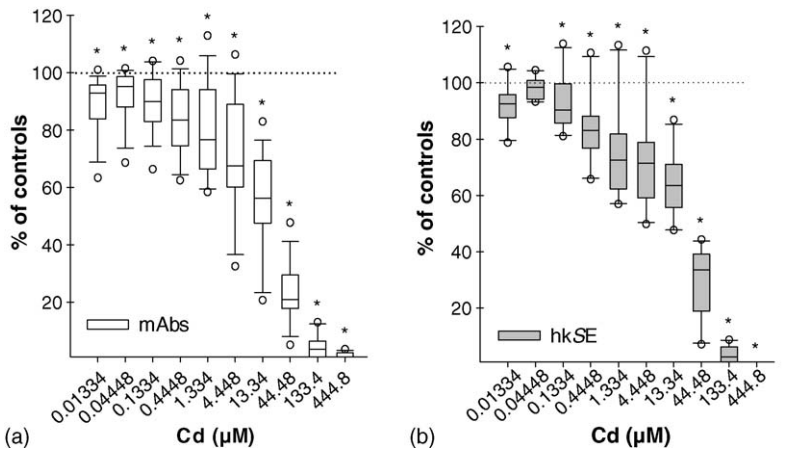

Fig. 1. The effect of cadmium (Cd) on the vitality and/or proliferation of (a) mAbs- and (b) hk*S*E-stimulated human PBMC. Medians and the 10th, 25th, 75th and 90th percentiles of 14 samples, three replicates each, are shown as percentages of the controls. The asterisks (*) indicate the significance as tested by the Wilcoxon test ($p = 0.05$).

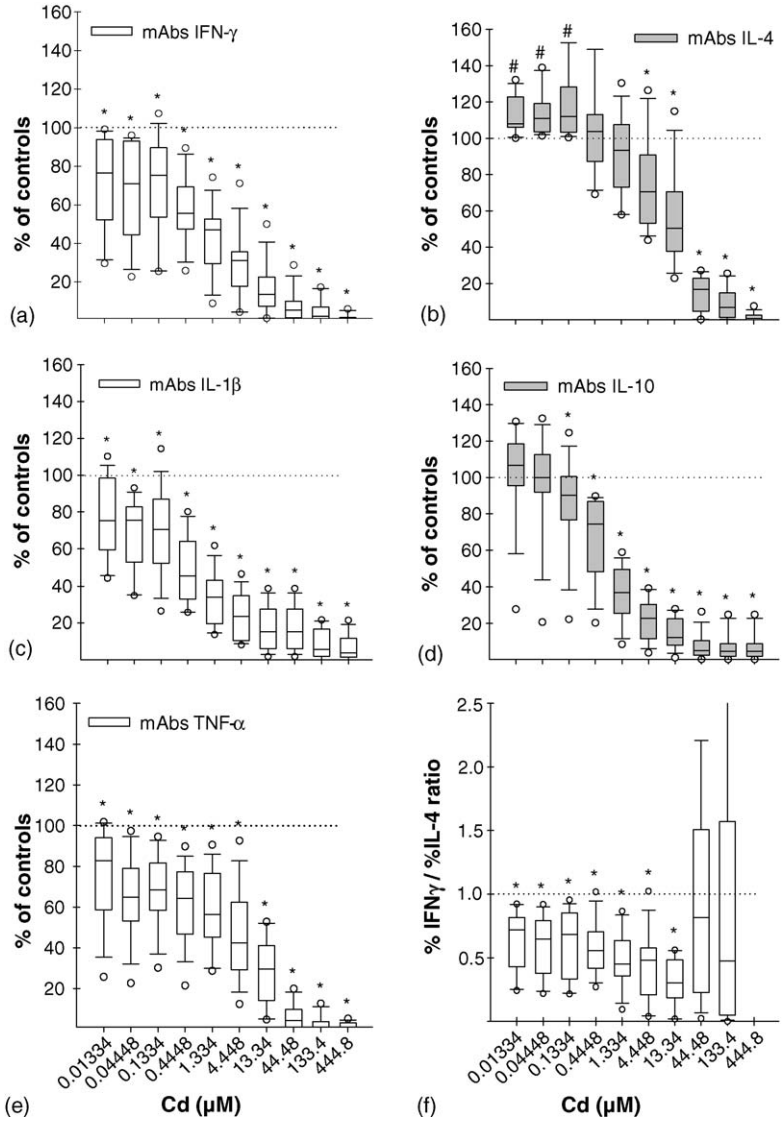

Fig. 2. The effect of cadmium (Cd) on the mAbs-stimulated cytokine release of (a) IFN-γ, (b) IL-4, (c) IL-1β, (d) IL-10, (e) TNF-α and (f) %IFN-γ/%IL-4 by human PBMC. Medians and the 10th, 25th, 75th and 90th percentiles ranges of 14 donors are shown as percentages of the controls. Symbols above each plot show whether, at each dose, cytokine release was significantly stimulated (#) or suppressed (*) compared with the controls ($p < 0.05$ in Wilcoxon test).

(Figs. 2 and 3) and gene expression (Fig. 4) of all measured cytokines. On the contrary, lower doses caused differential effects on the in vitro cytokine profiles. In response to antibody stimulation, secretion of the proinflammatory cytokines IL-1β and TNF-α as well as the type-1 T helper (T_H)1 cytokine IFN-γ was found to be highly reduced due to exposure to low Cd doses more than that of T_H2 cytokines IL-4 and -10 (Fig. 2). In this case, estimating the ratio %IFN-γ/%IL-4 (Fig. 2f) revealed a polarized type-2 immune response at all tested doses.

These results could be also confirmed by investigating cytokine gene expression. As shown in Fig. 4a, expression of IFN-γ gene was decreased in all samples tested at doses from 133 nM to 13.3 μM. Interleukin-4 gene expression, however, was enhanced at lower doses from 13.3 to 133 nM and decreased at higher doses. As a result, the ratio %IFN-γ/%IL-4 gene expression (Fig. 4b) was significantly decreased at most tested doses.

In cells stimulated with hk*S*E, levels of IL-4 were undetectable. In contrast to mAb-stimulation, IL-10 secretion was relatively more inhibited compared to

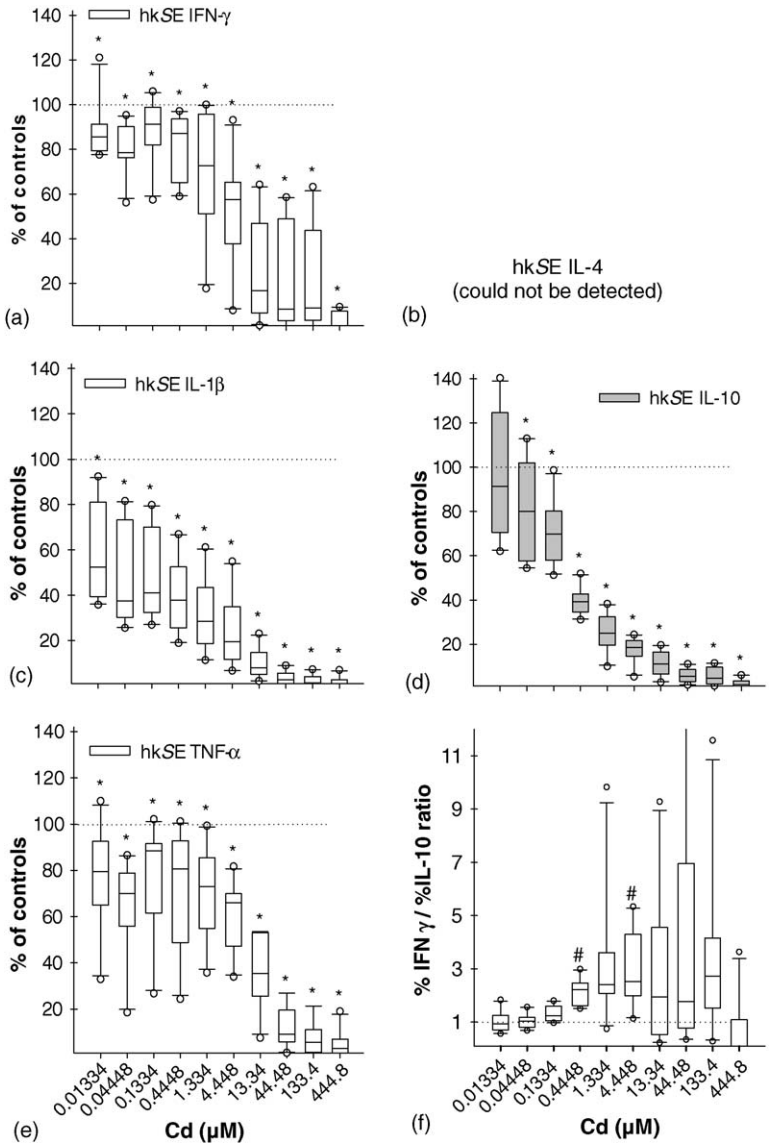

Fig. 3. The effect of cadmium (Cd) on the hk*S*E-stimulated cytokine release of (a) IFN-γ, (b) IL-4, (c) IL-1β, (d) IL-10, (e) TNF-α and (f) %IFN-γ/%IL-10 by human PBMC. Medians and the 10th, 25th, 75th and 90th percentiles ranges of 10 donors are shown as percentages of the controls. Symbols above each plot show whether, at each dose, cytokine release was significantly stimulated (#) or suppressed (*) compared with the controls ($p < 0.05$ in Wilcoxon test).

secretion of IFN-γ and TNF-α (Fig. 3a, b and e). Therefore, the ratio %IFN-γ/%IL-10 in the case of hk*S*E (Fig. 4f) was reversed compared to the mAb-stimulation and revealed a polarization tendency toward type-1 immune response.

4. Discussion

Although there have been some reports concerning the effects of Cd exposure on immune responses and cytokine production, reviewing these studies has revealed many inconsistencies, which may be, at least in part, due to the different experimental conditions used including different application regimes or varying animal models. These inconsistencies, in addition to the very long biological half-life of Cd, which ranges from 10 to 30 years (Järup et al., 1998) and the increasing levels of human Cd exposure in some countries, e.g. estimated dietary Cd intake of 10–30 μg/day in Europe (Nasreddine and Parent-Massin, 2002), exposure levels

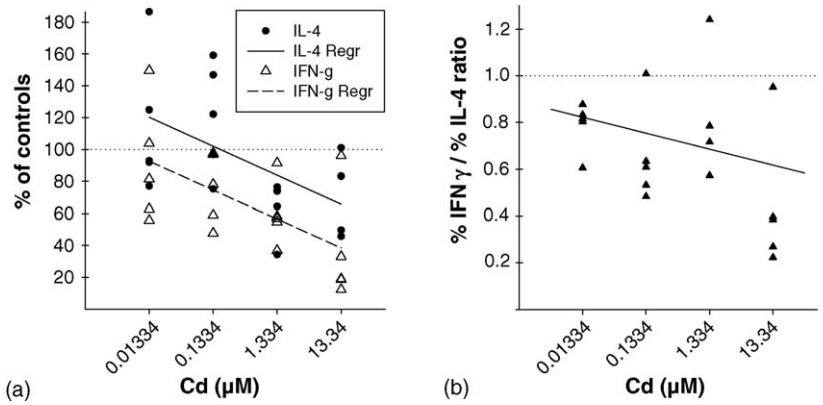

Fig. 4. The effect of cadmium (Cd) on cytokine gene expression of mAbs-stimulated PBMC as measured by real-time RT-PCR. Results of five donors are shown for both IL-4 and IFN-γ as percentages of the controls (a). Regression (Regr) lines are shown for both genes. To indicate the ratio T_H1/T_H2, %IFN-γ/%IL-4 are represented (b).

of 30–50 μg/day in Australia (Satarug et al., 2003) or even up to 240 μg/day dietary intake in Egypt (Saleh et al., 1998), underscores the need for further studies.

Disturbances in cytokine synthesis can be best studied under dynamic conditions by stimulating competent cells and analysing the patterns of cytokine production (De Groote et al., 1992), which may be differentially affected by heavy metals at concentrations not affecting other parameters (Shen et al., 2001). The present study confirms previous reports and reveals new insights into the effects of Cd on the immune system. Through evaluating cell vitality and cytokine responses, we demonstrated that while higher Cd doses were toxic, exposure to lower doses (as low as 0.013 μM) exerts immunomodulatory effects. As reviewed elsewhere (Beyersmann and Hechtenberg, 1997; Satoh et al., 2003), detailed studies have attributed cytotoxic properties of Cd to its interactions with cell receptors, and its accumulation intracellularly, which perturbs signal transduction and causes cell apoptosis or necrosis at lower and higher doses, respectively. Modification of Ca^{2+}-dependent processes (Beyersmann and Hechtenberg, 1997; Satoh et al., 2003) or cell cycle regulation factors (Zhou et al., 2004), and decrease in cellular content of glutathione (Figueiredo-Pereira et al., 1998; Nishimura et al., 2005) have been discussed as possible mechanisms for Cd-mediated cell death or impairment of cell functions. Taken together, it is likely that the intracellular Cd itself plays a critical role in the Cd-induced cytotoxicity as proposed by Nishimura et al. (2005), but a possible direct interaction between Cd ion and cell stimulants and/or cell receptors can not be ruled out. Cd ions can also contribute to the decreased cell vitality response at non-toxic doses of Cd. Thus in the light of our results, which are consistant with previous studies (Satoh et al., 2003), Cd has the ability to reduce cell vitality at low doses and is toxic at higher doses, but its in vivo toxicokinetics warrants further studies.

4.1. Discrepancies in documented effects of cadmium on the immune system

Our results showed that exposure to low and moderate Cd doses resulted in a clear bias of the immune response toward type-1 or type-2 depending on the activation method applied. Monoclonal antibody-activated cells exposed to Cd showed a significant increase in both IL-4 and -10 release, and a significant decrease in IFN-γ production. Release of the proinflammatory cytokines IL-1β and TNF-α was significantly reduced. Moreover, the immunomodulatory effect of Cd could be also demonstrated at the molecular RNA level supporting the suggestion that the suppression of IFN-γ production together with the increase of IL-4 release may be due to altered regulation of cytokine gene expression, and modulation of cytokine production potency. As expected, we obtained different results from cells activated with hk*S*E. Although the production of IL-4 could not be detected, IL-10 release was decreased at nearly all tested doses and was accompanied by a relative increase in IFN-γ production in comparison to mAb-activated cells.

There have been some conflicting results in the effect of Cd exposure on various immune components. Despite the well-documented effects (Satoh et al., 2003; Satarug and Moore, 2004), some studies demonstrated only negligible influences of Cd on the immune response. Chronic Cd feeding of 5 mg/kg body wt up to 7 weeks in rats (Chopra et al., 1984b) and for 2–6 months in the

primate Rhesus monkeys, (Chopra et al., 1985) did not result in any signs of immunomodulation, although Cd in the kidney was close to the toxic limit for man: 200 μg/g (W.H.O., 1977). Furthermore, proliferation was insignificantly increased in these monkeys. Similarly, chronic exposure of $B6C3F_1$ mice to 10–250 μg/ml of $CdCl_2$ for 90 days, revealed no changes in humoral immunity even though Cd had accumulated in significant quantities in the tissues (Thomas et al., 1985); macrophage bactericidal activity and delayed type hypersensitivity reactions were not significantly altered. All of these studies are more or less inconveniently non-conform, at least according to the conditions and doses adopted in our study, in particular in the case of Rhesus monkeys (Chopra et al., 1985), or they lacked the appropriate method to detect the modulation of the immune response.

Interestingly, Hilbertz et al. (1986) found that 1- and 20-h in vitro exposure of mouse macrophages to Cd increased or decreased the oxidative metabolism, respectively. The authors attributed the short-term effect to alterations in cell membrane, and suggested that longer exposures allow for greater metal uptake and more intracellular effects. Similar results have been reported by Zelikoff et al. (1995) who studied the immunotoxicity of Cd in trout fish, and speculated that discrepancies between the in vivo and in vitro studies may be due to differences in the site of metal action, degree of metal uptake and/or intracellular bioavailability.

Studies supporting a role of Cd in immune modulation demonstrated that heavy metals can increase the susceptibility of affected individuals to bacterial and viral infections (Shen et al., 2001; Simonyte et al., 2003). This has been attributed to suppression of the phagocytic activities (Loose et al., 1978; Goering et al., 2000), defect of macrophage recruitment to sites of infection (Simonet et al., 1984), suppression of NK cell activity (Chowdhury and Chandra, 1989), or the general suppression of humoral and cell-mediated immune responses (Dan et al., 2000).

Our results, indicating a bias towards type-2 immune response, are in agreement with previous data (Krocova et al., 2000). The latter authors reported that Cd at a dose of 20 μg/ml (177.9 μM) preferentially enhances the proliferation of murine T_H2 cells activated ex vitro by Con A. Our data highlight the similarity in reactivity between mice and human as previously suggested by Lawrence and McCabe (2002). Theocharis et al. (1991) observed a decrease in IL-2 and IFN-γ in presence of 10^{-4} M Cd, although Yucesoy et al. (1997) found that occupational exposure to Cd caused a significant increase in blood Cd levels with a significant suppression of the serum IL-1β but with no changes in IL-2 or TNF-α levels. These differences in reactivity of IFN-γ and TNF-α may result from other cross-regulatory processes of the immune system, which are compromised in our model. However, the finding that IL-10, produced by T_H2 cells, inhibits cytokine production by T_H1 cells (Fiorentino et al., 1989; Mosmann and Moore, 1991) and the dichotomy of T_H1 and T_H2 (Locksley et al., 1991; Dubey et al., 1991) confirms our results of lowered IFN-γ and TNF-α levels during the preferential proliferation of T_H2 in cells activated by monoclonal antibodies. Thus, short-term exposure of monoclonal antibody-stimulated cells to low Cd doses may cause a shift of the immune response from type-1 to -2. This may increase the incidence of allergic diseases (Mulligan et al., 2000; Oettgen and Geha, 2001; Lawrence and McCabe, 2002). In contrast to the stimulation by monoclonal antibodies, the stimulation of human PBMC by bacterial antigens resulted in higher T_H1/T_H2 ratios due to the relative higher IFN-γ production. Thus, response to bacterial antigens may overcome the shift into a type-2 response, and type-1 defence mechanisms appear not to be affected. Therefore, the immunomodulative effects of Cd, at least according to our model, may not affect the natural immune response against bacterial antigens.

The levels of Cd we applied in this study were in the range of the biologically relevant human exposure (Staessen et al., 2000; Nordberg, 2004) and up to toxic levels. We conclude that exposure of activated T-cells to low Cd doses leads to suppression of early T_H1 cytokine events, and stimulation of the T_H2 cytokines IL-4 and/or IL-10. This type-2-biased immune response may raise the possibility of Cd-promoted allergic diseases and clarify previous data on impaired inactivation of viruses and bacteria following exposure to Cd. Thus, our findings indicate that exposure even to low relevant Cd concentrations may have hazardous health consequences. This has to be proven by further in vivo and epidemiological studies.

Acknowledgments

We thank Katrin Bauer (IKIT, University of Leipzig, Germany) and Sylke Drubig (UFZ, Department of Environmental Immunology) for technical assistance and Dr. Sonya Faber (Coordination Centre for Clinical Trials, Leipzig, Germany) for reviewing the manuscript. This study was supported by a grant from the Interdisziplinäres Zentrum für Klinische Forschung (IZKF) of the Medical Faculty, University of Leipzig, Germany (project Z10).

References

Banerjee, S., Flores-Rozas, H., 2005. Cadmium inhibits mismatch repair by blocking the ATPase activity of the MSH2–MSH6 complex. Nucleic Acids Res. 33, 1410–1419.

Bench, G., Corzett, M.H., Martinelli, R., Balhorn, R., 1999. Cadmium concentrations in the testes, sperm, and spermatids of mice subjected to long-term cadmium chloride exposure. Cytometry 35, 30–36.

Beyersmann, D., Hechtenberg, S., 1997. Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicol. Appl. Pharmacol. 144, 247–261.

Borella, P., Giardino, A., 1991. Lead and cadmium at very low doses affect in vitro immune response of human lymphocytes. Environ. Res. 55, 165–177.

Borgman, R.F., Au, B., Chandra, R.K., 1986. Immunopathology of chronic cadmium administration in mice. Int. J. Immunopharmacol. 8, 813–817.

Cai, Y., Aoshima, K., Katoh, T., Teranishi, H., Kasuya, M., 2001. Renal tubular dysfunction in male inhabitants of a cadmium-polluted area in Toyama, Japan — an eleven-year follow-up study. J. Epidemiol. 11, 180–189.

Chopra, R.K., Kohli, K.K., Nath, R., 1984a. Effect of dietary chronic cadmium exposure on cell-mediated immune response in rhesus monkey (*Macaca mulatta*). Toxicol. Lett. 23, 99–107.

Chopra, R.K., Sehgal, S., Nath, R., 1984b. Cadmium an inhibitor of lymphocyte transformation and stimulator of antibody-dependent cell-mediated cytotoxicity (ADCC) in rats: the role of zinc. Toxicology 33, 303–310.

Chopra, R.K., Sharma, M., Nath, R., 1985. A nonimmunomodulatory effect of chronic cadmium exposure on lymphocyte transformation response in rhesus monkeys (*Macaca mulatta*). Exp. Cell Biol. 53, 19–23.

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.

Chowdhury, B.A., Friel, J.K., Chandra, R.K., 1987. Cadmium-induced immunopathology is prevented by zinc administration in mice. J. Nutr. 117, 1788–1794.

Dan, G., Lall, S.B., Rao, D.N., 2000. Humoral and cell mediated immune response to cadmium in mice. Drug Chem. Toxicol. 23, 349–360.

Daniels, M.J., Menache, M.G., Burleson, G.R., Graham, J.A., Selgrade, M.K., 1987. Effects of $NiCl_2$ and $CdCl_2$ on susceptibility to murine cytomegalovirus and virus-augmented natural killer cell and interferon responses. Fund. Appl. Toxicol. 8, 443–453.

De Groote, D., Zangerle, P.F., Gevaert, Y., Fassotte, M.F., Beguin, Y., Noizat-Pirenne, F., Pirenne, J., Gathy, R., Lopez, M., Dehart, I., 1992. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN-gamma and GM-CSF) in whole blood. Part I. Comparison with isolated PBMC stimulation. Cytokine 4, 239–248.

Dubey, C., Bellon, B., Druet, P., 1991. T_H1 and T_H2 dependent cytokines in experimental autoimmunity and immune reactions induced by chemicals. Eur. Cytokine Netw. 2, 147–152.

Fahmy, M.A., Aly, F.A., 2000. In vivo and in vitro studies on the genotoxicity of cadmium chloride in mice. J. Appl. Toxicol. 20, 231–238.

Figueiredo-Pereira, M.E., Yakushin, S., Cohen, G., 1998. Disruption of the intracellular sulfhydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. J. Biol. Chem. 273, 12703–12709.

Fiorentino, D.F., Bond, M.W., Mosmann, T.R., 1989. Two types of mouse T helper cell. Part IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J. Exp. Med. 170, 2081–2095.

Goering, P.L., Kuester, R.K., Neale, A.R., Chapekar, M.S., Zaremba, T.G., Gordon, E.A., Hitchins, V.M., 2000. Effects of particulate and soluble cadmium species on biochemical and functional parameters in cultured murine macrophages. In Vitro Mol. Toxicol. 13, 125–136.

Hemdan, N.Y.A., Emmrich, F., Adham, K., Wichmann, G., Lehmann, I., El Massry, A., Ghoneim, H., Lehmann, J., Sack, U., 2005. Dose-dependent modulation of the in vitro cytokine production of human immune competent cells by lead salts. Toxicol. Sci. 86, 75–83.

Hilbertz, U., Kramer, U., De Ruiter, N., Baginski, B., 1986. Effects of cadmium and lead on oxidative metabolism and phagocytosis by mouse peritoneal macrophages. Toxicology 39, 47–57.

Horiguchi, H., Harada, A., Oguma, E., Sato, M., Homma, Y., Kayama, F., Fukushima, M., Matsushima, K., 2000. Cadmium-induced acute hepatic injury is exacerbated in human interleukin-8 transgenic mice. Toxicol. Appl. Pharmacol. 163, 231–239.

Hovland Jr., D.N., Cantor, R.M., Lee, G.S., Machado, A.F., Collins, M.D., 2000. Identification of a murine locus conveying susceptibility to cadmium-induced forelimb malformations. Genomics 63, 193–201.

Järup, L., Berglund, M., Vahter, M., Elinder, C.G., Nordberg, G., 1998. Health effects of cadmium exposure — a review of the literature and a risk estimate. Scand. J. Work Environ. Health 24, 1–51.

Kim, S.D., Moon, C.K., Eun, S.Y., Ryu, P.D., Jo, S.A., 2005. Identification of ASK1, MKK4, JNK, c-Jun, and caspase-3 as a signaling cascade involved in cadmium-induced neuronal cell apoptosis. Biochem. Biophys. Res. Commun. 328, 326–334.

Krocova, Z., Macela, A., Kroca, M., Hernychova, L., 2000. The immunomodulatory effect(s) of lead and cadmium on the cells of immune system in vitro. Toxicol. In Vitro 14, 33–40.

Lawrence, D.A., McCabe Jr., M.J., 2002. Immunomodulation by metals. Int. Immunopharmacol. 2, 293–302.

Leffel, E.K., Wolf, C., Poklis, A., White, J., 2003. Drinking water exposure to cadmium, an environmental contaminant, results in the exacerbation of autoimmune disease in the murine model. Toxicology 188, 233–250.

Locksley, R.M., Heinzel, F.P., Holaday, B.J., Mutha, S.S., Reiner, S.L., Sadick, M.D., 1991. Induction of Th1 and Th2 CD4+ subsets during murine *Leishmania* major infection. Res. Immunol. 142, 28–32.

Loose, L.D., Silkworth, J.B., Simpson, D.W., 1978. Influence of cadmium on the phagocytic and microbicidal activity of murine peritoneal macrophages, pulmonary alveolar macrophages, and polymorphonuclear neutrophils. Infect. Immun. 22, 378–381.

Marth, E., Barth, S., Jelovcan, S., 2000. Influence of cadmium on the immune system. Description of stimulating reactions. Cent. Eur. J. Public Health 8, 40–44.

Mondal, T.K., Li, D., Swami, K., Dean, J.K., Hauer, C., Lawrence, D.A., 2005. Mercury impairment of mouse thymocyte survival in vitro: involvement of cellular thiols. J. Toxicol. Environ. Health A 68, 535–556.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65, 55–63.

Mosmann, T.R., Moore, K.W., 1991. The role of IL-10 in crossregulation of T_H1 and T_H2 responses. Immunol. Today 12, A49–A53.

Mulligan, M.S., Warner, R.L., McDuffie, J.E., Bolling, S.F., Sarma, J.V., Ward, P.A., 2000. Regulatory role of Th-2 cytokines, IL-10 and IL-4, in cardiac allograft rejection. Exp. Mol. Pathol. 69, 1–9.

Nasreddine, L., Parent-Massin, D., 2002. Food contamination by metals and pesticides in the European Union. Should we worry? Toxicol. Lett. 127, 29–41.

Nishimura, Y., Yamaguchi, J.Y., Kanada, A., Horimoto, K., Kanemaru, K., Satoh, M., Oyama, Y., 2005. Increase in intracellular Cd(2+) concentration of rat cerebellar granule neurons incubated with cadmium chloride: cadmium cytotoxicity under external Ca(2+)-free condition. Toxicol. In Vitro.

Nordberg, M., 2004. Environmental exposure and preventive measures in Sweden and EU. Biometals 17, 589–592.

Oettgen, H.C., Geha, R.S., 2001. IgE regulation and roles in asthma pathogenesis. J. Allergy Clin. Immunol. 107, 429–440.

Othumpangat, S., Kashon, M., Joseph, P., 2005. Eukaryotic translation initiation factor 4E is a cellular target for toxicity and death due to exposure to cadmium chloride. J. Biol. Chem. 280, 25162–25169.

Pillet, S., Rooney, A.A., Bouquegneau, J.M., Cyr, D.G., Fournier, M., 2005. Sex-specific effects of neonatal exposures to low levels of cadmium through maternal milk on development and immune functions of juvenile and adult rats. Toxicology 209, 289–301.

Sahmoun, A.E., Case, L.D., Jackson, S.A., Schwartz, G.G., 2005. Cadmium and prostate cancer: a critical epidemiologic analysis. Cancer Invest. 23, 256–263.

Saleh, Z.A., Brunn, H., Paetzold, R., Hussein, L., 1998. Nutrients and chemical residues in an Egyptian total mixed mixed diet. Food Chem. 63, 535–541.

Satarug, S., Moore, M.R., 2004. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ. Health Perspect. 112, 1099–1103.

Satarug, S., Baker, J.R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P.E.B., Williams, D.J., Moore, M.R., 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol. Lett. 137, 65–83.

Satoh, M., Kaji, T., Tohyama, C., 2003. Low dose exposure to cadmium and its health effects. Part 3. Toxicity in laboratory animals and cultured cells. Nippon Eiseigaku Zasshi 57, 615–623.

Satoh, M., Koyama, H., Kaji, T., Kito, H., Tohyama, C., 2002. Perspectives on cadmium toxicity research. Tohoku J. Exp. Med. 196, 23–32.

Schulte, S., Mengel, K., Gatke, U., Friedberg, K.D., 1994. No influence of cadmium on the production of specific antibodies in mice. Toxicology 93, 263–268.

Shen, X., Lee, K., Konig, R., 2001. Effects of heavy metal ions on resting and antigen-activated CD4(+) T cells. Toxicology 169, 67–80.

Shimada, H., Funakoshi, T., Waalkes, M.P., 2000. Acute, nontoxic cadmium exposure inhibits pancreatic protease activities in the mouse. Toxicol. Sci. 53, 474–480.

Simonet, M., Berche, P., Fauchere, J.L., Veron, M., 1984. Impaired resistance to Listeria monocytogenes in mice chronically exposed to cadmium. Immunology 53, 155–163.

Simonyte, S., Cerkasin, G., Planciuniene, R., Naginiene, R., Ryselis, S., Ivanov, L., 2003. Influence of cadmium and zinc on the mice resistance to Listeria monocytogenes infection. Medicina (Kaunas.) 39, 767–772.

Staessen, J.A., Kuznetsova, T., Roels, H.A., Emelianov, D., Fagard, R., 2000. Exposure to cadmium and conventional and ambulatory blood pressures in a prospective population study. Public Health and Environmental Exposure to Cadmium Study Group. Am. J. Hypertens. 13, 146–156.

Theocharis, S., Margeli, A., Panayiotidis, P., 1991. Effects of various metals on DNA synthesis and lymphokines production by human peripheral blood lymphocytes in vitro. Comp. Biochem. Physiol. C 99, 131–133.

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.

W.H.O., 1977. Task Group: Environmental Health Aspects of Cadmium. World Health Organization, Geneva.

Weggler, K., McLaughlin, M.J., Graham, R.D., 2004. Effect of chloride in soil solution on the plant availability of biosolid-borne cadmium. J. Environ. Qual. 33, 496–504.

Yucesoy, B., Turhan, A., Ure, M., Imir, T., Karakaya, A., 1997. Effects of occupational lead and cadmium exposure on some immunoregulatory cytokine levels in man. Toxicology 123, 143–147.

Zelikoff, J.T., Bowser, D., Squibb, K.S., Frenkel, K., 1995. Immunotoxicity of low level cadmium exposure in fish: an alternative animal model for immunotoxicological studies. J. Toxicol. Environ. Health 45, 235–248.

Zhou, T., Jia, X., Chapin, R.E., Maronpot, R.R., Harris, M.W., Liu, J., Waalkes, M.P., Eddy, E.M., 2004. Cadmium at a non-toxic dose alters gene expression in mouse testes. Toxicol. Lett. 154, 191–200.