Physiol. Res. 47: 453–461, 1998

Granulocyte and Plasma Cytokine Activity in Acute Cadmium Intoxication in Rats

M. KATARANOVSKI, D. KATARANOVSKI[1], D. SAVIC[1], G. JOVČIC[2], Z. BOGDANOVIC[3], T. JOVANOVIC[1].

Institute for Medical Research, Military Medical Academy, Belgrade, [1]Department of Ecology, Institute for Biological Research "Siniša Stankovic", [2]Institute of Medical Research and [3]Institute for Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Yugoslavia

Received April 1, 1998
Accepted July 29, 1998

Summary

Changes in the number and *ex vivo* function of peripheral blood neutrophils were investigated following intraperitoneal administration of cadmium-chloride in rats. Besides a dose-dependent increase in the number of peripheral blood neutrophils, changes were found in the functional state of isolated polymorphonuclear leukocytes (PMNs). Increased spontaneous adhesion and activation, and TNF activity in a conditioned medium were observed in cultures of granulocytes in comparison to granulocytes from control (saline-treated) animals. Increased levels of plasma activity of inflammatory cytokines, tumor necrosis factor (TNF) and interleukin-6 (IL-6) were noted following cadmium administration. Cytological signs of pulmonary inflammation were revealed histologically and the majority of neutrophils recovered from the lungs by enzyme digestion exhibited a capacity of nitroblue tetrazolium (NBT) reduction. Our data demonstrate that acute cadmium intoxication leads to a systemic inflammatory response characterized by numerical and functional changes in the granulocyte compartment and to increased levels of inflammation-related cytokine activity in the circulation. Correlations between the increased number of peripheral blood neutrophils and IL-6 plasma activity ($r=0.776$, $p<0.00001$) and the number of neutrophils recovered from the lung tissue ($r=0.893$, $p<0.00001$) suggested that systemic cadmium-induced inflammation might be involved in the pulmonary toxicity of cadmium.

Key words
Cadmium – Granulocytes – Cytokines – Inflammation

Introduction

Cadmium is one of the most toxic metals in our environment and its health effects have attracted much attention. The toxicity of cadmium has been established by histological studies in which damage primarily to the liver and kidneys, as well as to lungs and the gastrointestinal tract, has been demonstrated following both acute and chronic exposure to this metal (Friberg *et al.* 1986). A common pathological finding in cadmium intoxication is the infiltration of leukocytes, particularly neutrophils, into the afflicted tissues during the acute phase, which suggests a relationship between cadmium-induced tissue damage and neutrophil infiltration. Granulocyte infiltration may be secondary to parenchymal as well as nonparenchymal cell

Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

necrosis, as suggested in cadmium hepatotoxicity (Kayama *et al.* 1995) and toxicity to the lungs following intratracheal instillation of cadmium (Hirano *et al.* 1988, Driscoll *et al.* 1992). An influx of activated inflammatory cells has been suggested as the principal contributor to injurious peroxidative events in the lungs *via* generation of oxidative species (Manca *et al.* 1994).

Low molecular weight glycoprotein inflammatory mediators, cytokines, are part of the pathophysiological responses to cadmium in the liver (Kayama *et al.* 1995) and their increased content in the circulation might influence the functional state and migratory behaviour of peripheral blood cells, thus greatly contributing to the development of pulmonary inflammation (Fujishima and Aikawa 1995).

Despite the abundant histopathological evidence of leukocyte infiltration as a cytological indicator of inflammation in the liver and lungs, there are sparse data concerning changes in peripheral blood leukocytes in cadmium intoxication, as well as changes in the systemic microenvironment, which might influence this cell compartment. Recent studies have demonstrated changes in the functional state of peripheral blood cells exposed to cadmium *in vitro*, which might be of relevance for leukocyte function in cadmium intoxication *in vivo*. The increased production of a potent neutrophil chemotactic and activating protein, interleukin-8, by peripheral blood mononuclear cells (Horiguchi *et al.* 1993) and an increase in active adherence properties of peripheral blood granulocytes exposed to cadmium *in vitro* have been demonstrated (Macia and Hernandez 1995, Hernandez and Macia 1996). However, data concerning changes in peripheral blood leukocytes following cadmium administration *in vivo* are lacking. Hence, on a model of acute cadmium-induced toxicity we have studied the numerical and functional alterations of peripheral blood granulocytes including changes in their number and some functional parameters which might be of relevance for their function in cadmium-mediated tissue toxicity. We have also measured changes in the levels of inflammatory cytokines tumour necrosis factor (TNF) and interleukin-6 (IL-6) activity in the blood circulation. Our results demonstrated that intraperitoneal cadmium administration resulted in a systemic inflammatory response, characterized by numerical and functional changes in peripheral blood granulocytes and elevated plasma levels of activity of TNF and IL-6. Leukocyte infiltration in the lung tissue was noted following cadmium administration. Correlations between some of the components of cadmium-induced systemic inflammation and cytological signs of pulmonary inflammation (i.e. numbers of neutrophils recovered from lung tissue by enzyme digestion) were demonstrated and these relationships are discussed in the light of pulmonary toxicity of cadmium.

Methods

The experiments described in this paper were performed in adherence to the NIH guidelines for the use of experimental animals, with permission of the Ethical Committee of our Institute. Male Dark August (DA) rats weighing 200–250 g (Breeding Facilities of the Institute for Medical Research, Military Medical Academy) were housed in an air-conditioned room at 25 °C on a 12-h light/dark cycle. Animals were provided pelleted food (Veterinary Institute, Subotica) and tap water *ad libitum*. Cadmium chloride (Serva, Feinbiochemica, Germany) was prepared in sterile pyrogen-free saline (Central Pharmacy, Military Medical Academy, Belgrade) and administered intraperitoneally (in a constant volume of 0.5 ml) in doses of 0.5 mg/kg, 1 mg/kg and 2 mg/kg body mass of cadmium, with control group receiving saline only.

Serum aspartate amino transferase (AST) and alanine aminotransferase (ALT) activity were determined with an autoanalyzer (Ciba Corning Express, Oberline, Ohio, USA) using commercially available reagents. Urine (24-hour) was collected from rats placed in individual metabolic cages immediately following cadmium administration. The concentrations of creatinine in the serum and daily urine were determined by an automatic multichannel analyzer (Beckman-Astra, Irvine, Ca, USA) with commercially supplied reagents. The glomerular filtration rate was equated to the clearance of endogenous creatinine calculated in ml/h/100 g of body mass. Urine osmolarity was determined by the cryoscopic method using a Rebling osmometer (Rebling, USA).

White blood cell counts (WBC) with white cell differential counts were determined 24 h after cadmium administration by using an improved haemocytometer (Neubauer, Germany). The differential WBC count was determined in dried blood smears stained by May-Grünwald-Giemsa protocol. In each slide 200 cells were microscopically counted and the percentages of cells were assessed.

Peripheral blood granulocytes were isolated from the heparinized blood by centrifugation on a gradient NycoPrep Animal 1007 according to the manufacturer's procedure (Nycomed AS, Oslo, Norway). The purity of cells showed more than 95 % granulocytes. Granulocyte adherence to the plastic was assessed by a modified colorimetric assay and expressed as absorbance at 650 nm as described by Oez *et al.* (1990). The activation of granulocytes adhering to the plastic was evaluated by the capacity of nitroblue tetrazolium (NBT) reduction to the formazan product and expressed as absorbance at 570 nm as described by Monboise *et al.* (1991).

Interleukin-6 (IL-6) activity was determined by B9 assay as described by Shalaby *et al.* (1989) in which

IL-6 activity was measured by testing the capacity of plasma samples to stimulate proliferation of IL-6-dependent murine hybridoma cell line B9 (kindly supplied by L.A. Aarden, Clinical Laboratory of Netherlands, Amsterdam). IL-6 activity was calculated from a standard curve obtained by recombinant human IL-6 (Genzyme, USA) and expressed as U/ml. The specificity of IL-6-induced proliferation was confirmed by neutralizing polyclonal anti-human IL-6 antibody kindly donated by Dr G. Bendixen (University Hospital Denmark) previously shown to cross-react with rat IL-6 (Kataranovski *et al.* 1992).

TNF activity was measured in L-929 mouse fibroblast cell line bioassay for TNF according to Meager *et al.* (1989) by measuring the cytotoxic activity of each plasma sample and a granulocyte-conditioned medium in the presence of actinomycin D. The optical density of stained viable cells solubilized by 0.1 N hydrochloric acid was determined with ELISA processor at 650 nm. The specificity of TNF-induced cytotoxicity was confirmed by neutralizing the anti-mouse TNF-α antibody (cross-reactive with rat TNF) (Pejnovic *et al.* 1995). TNF activity was calculated from the standard curve of recombinant human TNF-α (Genzyme, USA) and expressed in pg/ml.

After exsanguination, the left lung lobe was removed and transverse sections were prepared of approximate equal size from each rat and fixed in buffered formaldehyde. Paraffin-embedded tissue sections were stained with haematoxylin and eosin. Samples of the right lobe were subjected to mild enzyme digestion by 0.5 % trypsin in phosphate-buffered saline (PBS) as described by Frangakis *et al.* (1982). Released cells were harvested every 15 min during one-hour digestion and inactivated with trypsin by adding foetal calf serum (FCS). Differential counts were performed on dried smears of harvested cells, stained according to the May-Grünwald-Giemsa protocol. Suspensions of harvested cells ($5x10^5$ cells in 100 ml per tube) were incubated with 100 ml of 2 mg/ml NBT in phosphate-buffered saline for 20 min at 37 °C, followed by 10 min at room temperature. The numbers of activated granulocytes were assessed by counting individual cells with a capacity to reduce nitroblue tetrazolium as described by Kataranovski *et al.* (1995). At least 300 polymorphonuclear cells with signs of intracellular and membrane reduction of NBT, visualised as fine dark dots of formazan, were counted under the light microscope at x 1000 magnification on dried smears stained by May-Grünwald-Giemsa protocol and NBT^+ granulocytes were scored. The results were expressed as the number of PMNs recovered per gram of wet lung mass.

Results are expressed as mean values ± S.E.M. for each experimental group or treatment, respectively. The significance between mean values was analysed by the Mann-Whitney U-test.

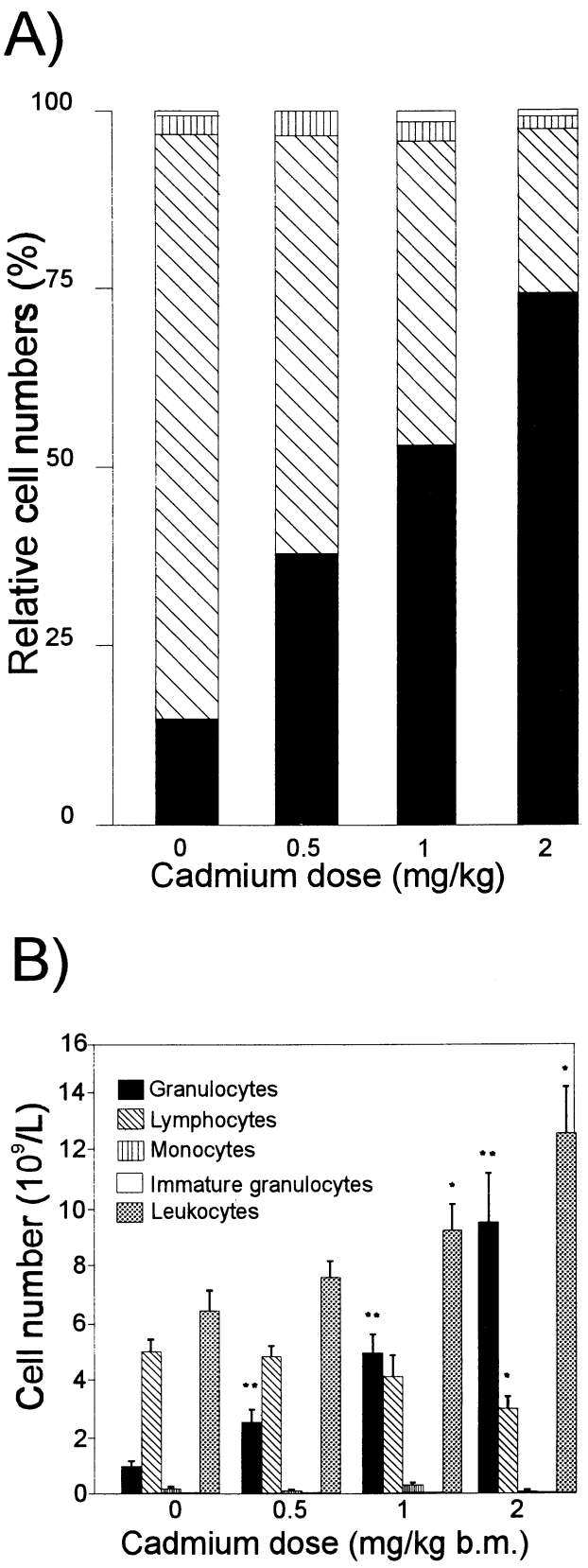

Fig. 1. *Differential relative (A) and total white blood cell counts (B) following cadmium administration. Six animals per group were used. Significantly different from controls (* $p<0.05$, ** $p<0.005$).*

Table 1. Changes of peripheral blood granulocyte function following cadmium administration

Function	Cadmium dose (mg/kg b.m.)			
	Controls	0.5	1.0	2.0
Adhesion	0.056±0.001	0.088±0.003**	0.082±0.008**	0.077±0.008**
Activation	0.046±0.002	0.045±0.002	0.074±0.003**	0.082±0.006**
TNF activity	97.46±25.16	270.04±14.8*	184.46±19.13*	47.9±8.0*

*Adhesion and activation expressed as absorbance at 650 nm and 570 nm, respectively. TNF activity expressed as pg/ml. Values given as means±SEM. Significantly different from controls at * $p<0.05$ and ** $p<0.01$ (Student's t-test).*

Results

Intraperitoneal administration of cadmium chloride led to leukocytosis in doses at 1 mg/kg b.m. and 2 mg/kg b.m. of cadmium ($p<0.05$ and $p<0.005$ vs control, respectively) and to changes of relative (Fig. 1A) and absolute (Fig. 1B) numbers of granulocytes in the peripheral blood. A progressive rise in the number of granulocytes occurred concomitantly with the decreased number of lymphocyte following increasing doses of cadmium. No changes in haematocrit values were noted after all doses of cadmium administered.

The changes in peripheral blood granulocyte function following cadmium administration are summarized in Table 1. Each result is expressed as the mean of quadruplicate determinations in a single preparation of neutrophils obtained from pooled heparinized blood of five animals. Similar changes were obtained in two separate series of experiments. Increased adhesion of granulocytes to polystyrene plastic of microtitre plate wells was noted following all the administered doses of cadmium, while increased NBT reduction was observed at doses 1 and 2 mg/kg b.m. of cadmium. Increased activity of tumour necrosis factor (TNF) was observed in media conditioned by granulocytes at 0.5 mg/kg b.m. and 1 mg/kg b.m. of administered cadmium in comparison to values from control samples, while the 2 mg/kg b.m. dose of cadmium decreased the levels of TNF activity in the granulocyte-conditioned medium.

A two- to three-fold increase in the levels of plasma TNF activity was observed following cadmium administration (Fig. 2). The 0.5 mg/kg b.m. dose increased plasma IL-6 activity which was followed by dramatically increased levels of activity of this cytokine after the 1 mg/kg b.m. dose of cadmium and persistently high levels of IL-6 activity after 2 mg/kg b.m. of cadmium.

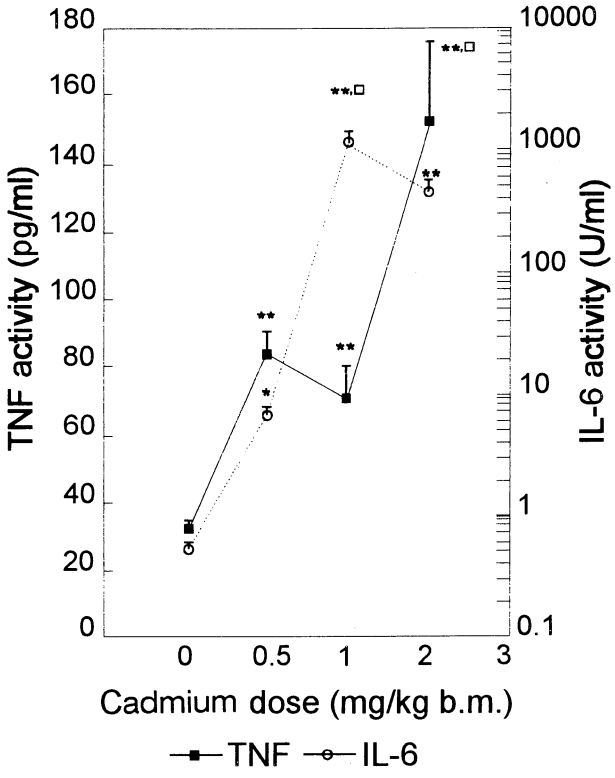

Fig. 2. *Plasma TNF and IL-6 activity following cadmium administration. Six animals per group were used. Data are expressed as pg/ml and U/ml of activity for TNF and IL-6, respectively. Significance at * $p<0.01$, ** $p<0.005$, open square $p<0.05$ vs value at lower cadmium dose.*

At all administered cadmium doses no changes in ALT, liver specific enzyme, were noted, but rise in AST, enzyme with the highest concentration in liver in comparison to other tissues, was noted at cadmium doses of 1 mg/kg b.m. and 2 mg/kg b.m.

(117.30±17.42 U/ml, $p<0.05$ and 184.78±31.03 U/ml, $p<0.001$ compared to 84.82±9.96 U/ml in the controls), suggesting cadmium toxicity to liver. Increase in urine osmolarity (2793±124 mOsm/kg, $p<0.05$ vs 1848±120 mOsm/kg in controls) and decrease in clearance of creatinine noted at cadmium dose of 0.5 mg/kg (18.41±2.29 ml/h/100 g b.m. $p<0.01$ vs 38.40±4.00 ml/h/100 g b.m. in control, saline received animals), as well as increased values of the clearance of creatinine at higher doses of cadmium (49.28±4.59 mOsm/kg and 52.57±2.96 mOsm/kg at 1 mg kg/b.m. and 2 mg/kg b.m., respectively, the latter value significant at $p<0.01$ vs controls) suggested changes in renal function.

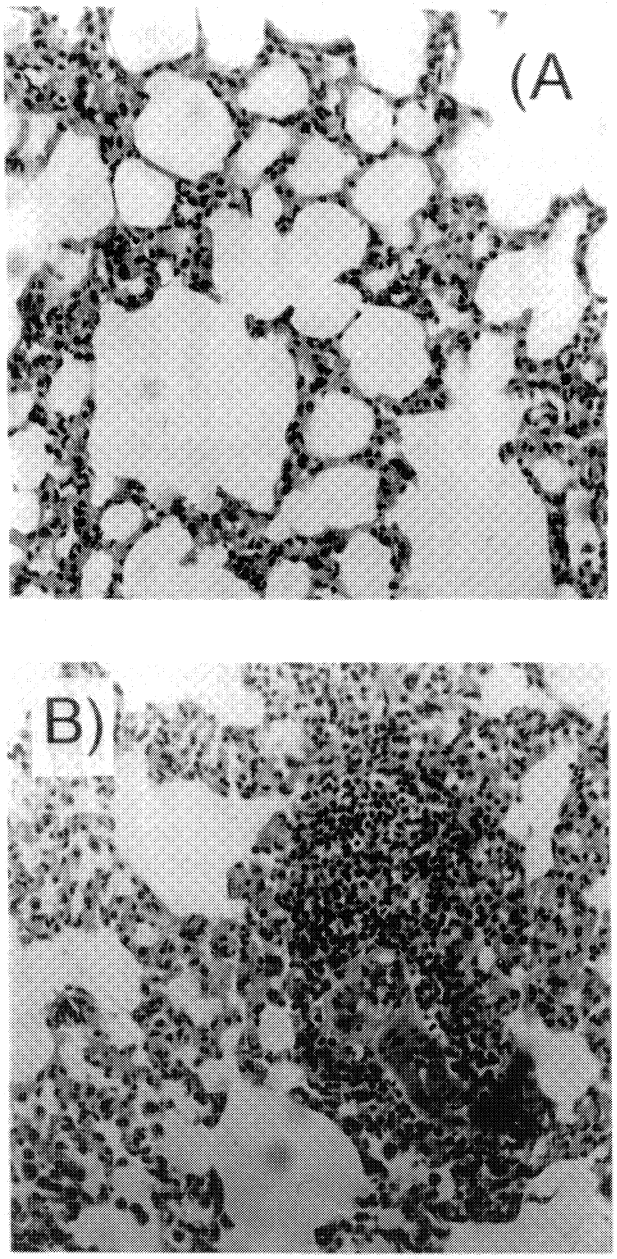

Fig. 3. *Lung histology and granulocyte recovery. Histological picture of lungs from rats which had received saline (3A) or 2 mg Cd/kg b.m. (3B). The numbers of recovered lung granulocytes and NBT^+ neutrophils following cadmium administration (3C) were obtained from six animals. Significantly different from controls (* $p<0.005$).*

The microscopical appearance of lungs with thin interalveolar walls, regular alveolar spaces without inflammatory infiltration was normal in control animals which had received saline only (Fig. 3A). The histological picture was different in rats which had received 0.5 mg Cd/kg. The lung parenchyma was collapsed, the interalveolar walls were thickened with extravasation of erythrocytes, pneumocyte desquamation in alveolar spaces, prominent clogging of arterioles and capillary spaces and erythrocyte extravasation. Further thickening of interalveolar walls with granulocytes was observed in rats with 1 mg Cd/kg dose. The inflammatory changes after the 2 mg Cd/kg dose were more prominent and were

accompanied by massive granulocyte infiltration (Fig. 3B). The granulocyte recovery from the lung tissue was enhanced. The majority of granulocytes exhibited an ability to reduce nitroblue tetrazolium, at cadmium doses of 1 mg/kg b.m. and 2 mg/kg b.m. (Fig. 3C).

Significant correlation ($r=0.776$, $p<0.0001$) was found between the cadmium dose-dependent rise in granulocyte numbers and the increase in plasma IL-6 activity (Fig. 4A). The respective regression formula was: $y = 0.443 + 2.434 \times$ log IL-6 activity, where y = granulocyte number. The numbers of granulocytes recovered from the lung tissue correlated significantly with the numbers of peripheral blood polymorphonuclear leukocytes (Fig. 4B) ($r=0.893$, $p<0.00001$) and the corresponding regression formula was $y = 0.039 + 0.267$ peripheral blood PMN numbers (y = recovered lung PMN numbers).

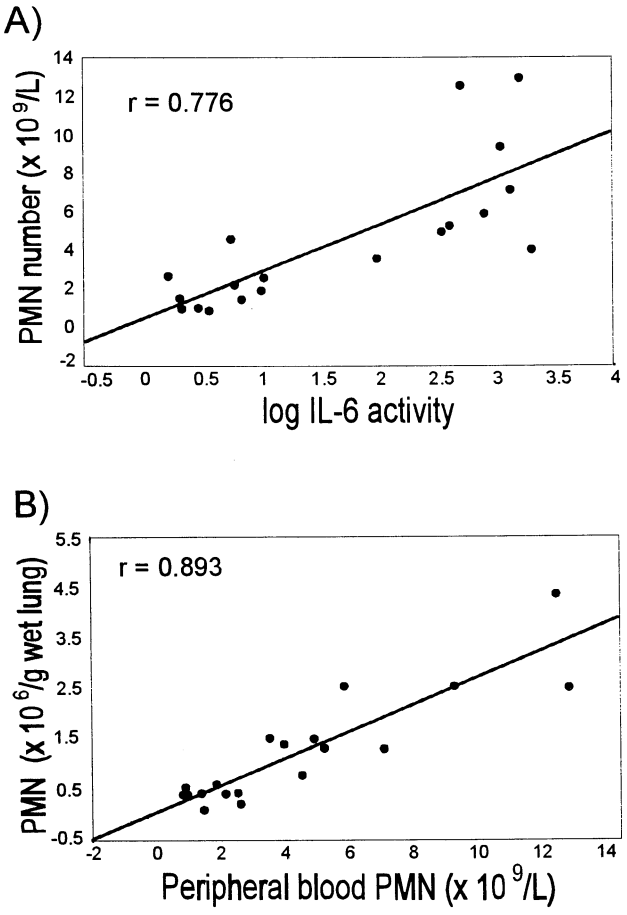

Fig. 4. *The regression line for peripheral blood PMN numbers and log IL-6 activity (A) and peripheral blood PMNs and PMNs recovered from lungs (B).*

Discussion

Intraperitoneal administration of various doses of cadmium to rats leads to a systemic inflammatory response characterized by leukocytosis, changes in the cell ratio and activity as well as by rise in plasma cytokine activity. The shift in the ratio of lymphocytes and neutrophils in favour of neutrophils in the peripheral circulation might be the result of increased demands for granulocytes at the site of local inflammation (cadmium deposition), as described for other toxic substances administered intraperitoneally (Jovčic *et al.* 1993). This response is accompanied by systemic inflammation characterized by a rise of plasma activity of inflammation related cytokines TNF and IL-6 which might influence the granulocyte compartment. Significant correlation ($r=0.776$, $p<0.0001$) between the increase in neutrophil numbers and changes of interleukin-6 plasma activity suggests that this cytokine plays a role in the granulocytosis during acute cadmium intoxication. This is in agreement with the *in vivo* induction of neutrophilia following recombinant IL-6 administration in rats, in which both the demarginating and myelopoietic effects have been proposed (Ulich *et al.* 1989).

The increased numbers of circulating neutrophils might be a result of reparation processes of haemopoiesis described in mice following cadmium administration (Macková *et al.* 1996). In addition, accelerated regeneration of granulocyte-macrophage progenitor cells observed in irradiated mice pretreated with cadmium (Fedoročko *et al.* 1996) would be of relevance for the increased number of circulating neutrophils detected in our study. The decreased numbers of lymphocytes might be the result of cadmium toxicity to lymphocytes (Exon and Kohler 1986) and/or the susceptibility of primary lymphoid organs to cadmium, e.g. the thymus, as reported previously in rats (Morselt *et al.* 1988).

In vitro tests of granulocyte function are useful tools for studying neutrophil functional state and have facilitated the analysis of their functional state *ex vivo*. Adhesiveness of granulocytes is their fundamental property and quantitation of active adherence capacity to plastic is recommended as an *in vitro* correlate of granulocyte adhesion to endothelium or connective tissue matrices (Oez *et al.* 1990). The *in vitro* potential of granulocytes to generate reactive oxygen intermediates *via* tetrazolium reducing respiratory burst oxidase (Baehner *et al.* 1976, Kakinuma *et al.* 1987) can be determined by measuring the reduction of tetrazolium salts such as NBT to formazan products and this granulocyte activity is employed as an *in vitro* measure of granulocyte activation (Monboise *et al.* 1991). Changes in the functional state of granulocytes *in vitro* (Table 1) following cadmium administration *in vivo* are in accordance with data which have demonstrated an increased metabolic activity of spleen phagocytes from mice which had received intraperitoneal cadmium chloride (Čiž *et al.* 1996). Increased granulocyte adherence to plastic could be the consequence of a direct action of injected cadmium as it stimulates early spontaneous adherence to plastic of

peripheral human polymorphonuclear leukocytes (Macia and Hernandez 1995) or it could be a consequence of elevated levels of cytokine activity in systemic circulation following cadmium administration. In this respect, a role of TNF and IL-6 in enhancing granulocyte activity relevant to their effector function in inflammation has been demonstrated (Kapp and Zeck-Kapp 1990, Yee and Christou 1994, Biffl *et al.* 1994). Elevated levels of TNF activity in granulocyte conditioned media are in line with the recently described capacity of these cells for cytokine production (Casatella 1995). This granulocyte activity is considered to be a part of their functional capacity to produce mediators involved in their effector functions. Granulocyte TNF production is probably the underlying mechanism of the regulation of local inflammation as proposed by Fujishima and Aikawa (1995). The decrease in TNF bioactivity in a granulocyte conditioned medium, demonstrated at highest doses of cadmium could be ascribed to the presence of TNF antagonists such as soluble TNF receptors. Neutrophil granulocytes possess specific receptors for TNF (Fujishima and Aikawa 1995), which might act as a competitive inhibitor for this cytokine when shed after an inflammatory stimulus as described for other TNF receptor-bearing cells in various inflammatory conditions (Eigler *et al.* 1997).

Increased levels of plasma TNF and IL-6 activity are indicators of inflammation at the systemic level (Ohzato *et al.* 1992) and might result from cadmium-induced cell necrosis, cell injury or cytokines from local inflammation in the peritoneal cavity. As these mediators could mobilize the metabolic response of the whole organism and modulate cell behaviour in inflammation, elevated plasma cytokine activity following intraperitoneal administration of cadmium might influence tissue response to cadmium administration. In this respect, involvement of TNF in the development of an acute inflammatory response characterized by a rise of acute phase reactants following subchronic intravenous cadmium administration in mice was implicated and its role in cadmium hepatotoxicity has been proposed (Kayama *et al.* 1995).

The microscopical appearance of lungs in our study suggested pulmonary toxicity in addition to hepatotoxicity and changes in renal function indicated by the clinical and biochemical findings. Cell infiltration and the enhanced activated (NBT^+) PMN recovery from the lungs demonstrated cellular signs of pulmonary inflammation, characteristic for the cytologic response following direct cadmium administration into lung tissue (Hirano *et al.* 1988, Driscoll *et al.* 1992). Development of pulmonary inflammation far from the site of cadmium administration was suggested in the same experimental model of acute cadmium toxicity in rats with lung protein recovery as a surrogate of pulmonary inflammation (Manca *et al.* 1994). It was hypothesized that acute toxicity of cadmium to lung tissue might represent a secondary consequence of the primary cadmium insult, with an influx of activated inflammatory cells the principal contributor to injurious peroxidative events (Manca *et al.* 1994). Leukocyte infiltration in lung tissue demonstrated in our study may be secondary to direct cadmium toxicity to parenchymal and/or nonparenchymal cells, as was suggested by the desquamation of alveolar epithelium (not shown), with further amplification of lung tissue injury by products of activated neutrophils. The increased numbers of neutrophils in the circulation might facilitate the development of cellular components of pulmonary inflammation. The close correlation between neutrophil numbers in peripheral blood and the number of cells recovered from lungs by enzyme digestion ($r=0.893$, $p<0.0001$) supports this assumption. The correlations were also demonstrated between levels of plasma IL-6 activity and numbers of granulocytes in peripheral blood ($r=0.776$, $p<0.00001$) and between granulocyte numbers in the circulation and those recovered from the lungs. Such observations suggested an interrelationship between these components of systemic inflammation in pulmonary cadmium toxicity.

In conclusion, our results show that cadmium induces a systemic inflammatory response with changes in the number and functional state of polymorphonuclear leukocytes and increased levels of inflammation-relevant cytokine activity. Correlation between some components of this response suggest that this systemic inflammatory response might be of relevance in pulmonary inflammation.

Acknowledgements

This study was supported by the Serbian Ministry of Sciences, Yugoslavia, Grant No. 03 E 15.

References

BAEHNER R.L., BOXER L.A., DAVIS J.: The biochemical basis of nitroblue tetrazolium reduction in normal human and chronic granulomatous disease polymorphonuclear leukocytes. *Blood* **48**: 309–313, 1976.

BIFFL W., MOORE E.E., MOORE F.A., CARL V.S., KIM F.J., FRANCOISE R.J.: Interleukin-6 potentiates neutrophil priming with platelet activating factor. *Arch. Surg.* **129**: 1131–1136, 1994.

CASATELLA M.A.: The production of cytokines by polymorphonuclear neutrophils. *Immunol. Today* **16**: 21–26, 1995.

ČÍŽ M., LOJEK A., HOŠEK B., FEILHAUEROVA M.: Cadmium ions influence the chemiluminiscence activity of murine splenocytes both *in vitro* and *in vivo*. *Toxicol. Lett.* **86:** 39–45, 1996.

DRISCOLL K.E., MAURER J.K., POYNTER J., HIGGINS J., ASQIUTH T., MILLER N.S.: Stimulation of rat macrophage fibronectin release in a cadmium chloride model of lung injury and fibrosis. *Toxicol. Appl. Pharmacol.* **116:** 30–37, 1992.

EIGLER A., SINHA B., HARTMANN G., ENDRES S.: Taming TNF: strategies to restrain this proinflammatory cytokine. *Immunol. Today* **18:** 487–492, 1997.

EXON J.H., KOHLER L.D.: Immunotoxicity of cadmium. In: *Cadmium*, E.C. FOULKERS (ed.), Springer, Berlin, 1986, pp. 339–348.

FEDOROČKO P., DOMONKOŠOVÁ A., KUNDRATOVÁ T., MACKOVÁ N.O., BREZÁNI P., FEDOROČKOVÁ A.: Effects of cadmium on haemopoiesis in irradiated and non-irradiated mice: 1. Relationship to the number of myeloid progenitor cells. *Physiol Res* **45:** 93–100, 1996.

FRANGAKIS M.V., KOOPMAN W.J., KIYONO H., MICHALEK S.M., MCGHEE J.R.: An enzymatic method for preparation of dissociated murine Peyer's patch cells enriched for macrophages. *J. Immunol. Meth.* **48:** 33–44, 1982.

FRIBERG L., KJELSTROM T., NORDBERG G.F.: Cadmium. In: *Handbook on the Toxicology of Metals*. L. FRIBERG, G.F. NORDBERG, V. VOUK (eds), Elsevier, Amsterdam, 1986, pp. 130–138.

FUJISHIMA S., AIKAWA N.: Neutrophil-mediated tissue injury and its modulation. *Intensive Care Med.* **21:** 277–285, 1995.

HIRANO S., TSUKAMOTO N., HIGO S., SUZUKI K.T.: Toxicity of cadmium oxide instilled into the rat lung. II. Inflammatory responses in broncho-alveolar lavage fluid. *Toxicology* **55:** 25–35, 1988.

HERNANDEZ M., MACIA M.: Free peripheral sulfhydril groups, CD11/CD18 integrins and calcium are required in the cadmium and nickel enhancement of human polymorphonuclear leukocyte adherence. *Arch. Environment. Contam. Toxicol.* **30:** 437–443, 1996.

HORIGUCHI H., MUKAIDA N., OKAMOTO S.I., TERANISHI H., KASUYA M., MATSUSHIMA K.: Cadmium induces interleukin-8 production in human peripheral blood mononuclear cells with the concomitant generation of superoxide radicals. *Lymphokine Cytokine Res.* **12:** 412–428, 1993.

JOVČIC G., STOJANOVIC N., KATARANOVSKI M., PETAKOV M., OBRADOVIC P.: Acute sterile inflammation: correlation between cellular changes and extramedullary produced regulators in vivo. *Ann. Hematol.* **66:** 195–201, 1993.

KAKINUMA K., FUKUHARA Y., KANEDA M:. The respiratory burst oxidase of neutrophils. *J. Biol. Chem.* **262:** 12316–12324, 1987.

KAPP A., ZECK-KAPP G.: Activation of the oxidative metabolism in human polymorphonuclear neutrophilic granulocytes: The role of immunomodulating cytokines. *J. Invest. Dermatol.* **95:** 94S–99S, 1990.

KATARANOVSKI M., KUČUK J., LILIC D., DRAŠKOVIC-PAVLOVIC B., ČOLIC M., PEJNOVIC N., DUJIC A.: Increased activity of lymph node cells in thermal injury. *Reg. Immunol.* **4:** 197–203, 1992.

KATARANOVSKI M., ČANČAR D., BOGDANOVIC Z., KATARANOVSKI D., JOVČIC G., PEJNOVIC N., DUJIC A.: Posttraumatic changes in lung compartment in two inbred strains of rats: evaluation by short-term organ culture. In: *Proceedings of the 8th Congress of Intensive Care Medicine*. C. ROUSSOS (ed.), Monduzzi Editore, 1995, pp. 709–712.

KAYAMA F., YOSHIDA T., EWELL M.R., LUSTER M.I.: Role of tumor necrosis factor in cadmium-induced hepatotoxicity. *Toxicol. Appl. Pharmacol.* **131:** 224–234, 1995.

MACIA M., HERNANDEZ M.: Modulation of the adherence of human polymorphonuclear leukocytes by cadmium and nickel: sexual differences. *Arch. Environment. Contam. Toxicol.* **29:** 15–19,1995.

MACKOVÁ N.O., LENÍKOVÁ S., FEDOROČKO P., BREZÁNI P., FEDOROČKOVÁ A.: Effects of cadmium on haemopoiesis in irradiated and non-irradiated mice: 2. Relationship to the number of circulating blood cells and haemopoiesis. *Physiol. Res.* **45:** 101–106, 1996.

MANCA D., RICARD A.C., TRA H.V., CHEVALIER G.: Relation between lipid peroxidation and inflammation in the pulmonary toxicity of cadmium. *Arch. Toxicol.* **68:** 364–369, 1994.

MEAGER A., LEUNG H., WOOLEY J.: Assays for tumor necrosis factor and related cytokines. *J. Immunol. Meth.* **116:** 1–17, 1989.

MONBOISE J., GARNOTEL R., RANDOUX A.: Adhesion of human neutrophils to and activation by type-I collagen involving a beta-2 integrin. *J. Leukoc. Biol.* **50:** 373–380, 1991.

MORSELT A.F., LEENE W., DE GROOT C., KIPP J.B., EVERS M., ROELOFSEN A.M., BOSCH K.S.: Differences in immunological susceptibility to cadmium toxicity between two rat strains as demonstrated with cell biological methods. *Toxicology* **48:** 127–139, 1988.

OEZ S., WELTE E., PLATZER E., KALDEN J.R.: A simple assay for quantifying the inducible adherence of neutrophils. *Immunobiology* **180:** 308–315, 1990.

OHZATO H., YOSHIZAKI K., NISHIMOTO N., OGATA A., TAGOH H., MONDEN M., GOTOH M., KISHIMOTO T., MORI T.: Interleukin-6 as a new indicator of inflammatory status. Detection of serum levels of Interleukin-6 and C-reactive protein after surgery. *Surgery* **111:** 201–209, 1992.

PEJNOVIC N., LILIC D., ŽUNIC G., ČOLIC M., KATARANOVSKI M., DUJIC A.: Aberrant levels of cytokines within the healing wound after burn injury. *Arch. Surg.* **130:** 999–1006, 1995.

SHALABY M.R., WAAGE A., AARDEN L., ESPEVIK T.: Endotoxin, tumor necrosis factor alpha and Interleukin-1 induce interleukin-6 production in vivo. *Clin. Immunol. Immunopathol.* **53:** 488–498, 1989.

ULICH T.R., DELCASTILLO J., GUO K.: In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. *Blood* **73:** 108–110, 1989.

YEE J., CHRISTOU N.V.: The local role of tumor necrosis factor alpha in the modulation of neutrophil function at sites of inflammation. *Arch. Surg.* **129:** 1249–1255, 1994.

Reprint requests

M. Kataranovski Ph.D., Senior Research Associate, Institute for Medical Research, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Yugoslavia.