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Differential effects of cadmium on blood lymphocyte subsets

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Abstract

This work was designed to analyze the possible dose dependent effects of cadmium on the blood lymphocyte subset distribution and if these effects are related to circulating cadmium concentration. For that purpose, adult male rats were exposed for one month to 0, 5, 10, 25, 50 or 100 ppm of cadmium chloride ($CdCl_2$) in the drinking water. B lymphocytes decreased in peripheral blood with the doses of 5 and 10 ppm of $CdCl_2$. From the dose of 25 ppm on, B cells increased. T lymphocytes were increased with the doses of 25, 50 and 100 ppm of $CdCl_2$. The lower doses of the metal induced opposite effects. $CD4^+$ and $CD8^+$ cells decreased with the doses of 5 and 10 ppm whereas they were increased with the dose of 25 ppm of $CdCl_2$ on. From the dose of 10 ppm on, cadmium concentration was increased. The results on the distribution of blood lymphocyte subsets suggest that cadmium inhibits the humoral and cellular immune response with the lower doses of the metal used, and opposite effects were detected with the higher doses, the effect not being dependent on the circulating cadmium.

Introduction

The sensitivity of the immune system to immunosuppression by an environmental agent is due as much to the general properties of the chemical as to the complex nature of the immune system. The immune system may be affected by cadmium, being the effects of this metal correlated either with an abnormal humoral or cellular responses, although, the data available are scant and in some extent controversial (Koller 1998). There are some criterions that have to be considered: mitogens assays do not provide a measure of immune function and an estimation of specific subpopulation has to be studied as some of them can be changed while others not. However, most reports studied mitogen stimulation (Borella & Giardino 1991; Snyder & Valle 1991; Szuster-Ciesielska *et al.* 2000) whereas others studies analyzed phenotypes (Yucesoy *et al.* 1997; Sarasua *et al.* 2000). Besides, simultaneous determinations of mitogen-stimulation and/or cytokines releases have been less studied (Petanova *et al.* 2000; Krocova *et al.* 2000). In general, host resistance and delayed hypersensitivity have been the usual tests to analyze the effects of the metal on the immune system (Ilback *et al.* 1994; Dan *et al.* 2000). The variability of the results reported may be due to the ability of cadmium to induce metalothionein synthesis as a function of the considered tissue that is genetically determined (Liu *et al.* 1999; Waalkes *et al.* 2000). Moreover, indirect effects of the metal mediated by the endocrine system (i.e. pituitary hormones) have to be considered (Sanders 1996; Lafuente & Esquifino 1999; Lall & Dan 1999). In fact, the effects of pituitary hormone like prolactin, GH or ACTH, affected by the metal, on the immune system is well documented (Lafuente *et al.* 2003a; Weigent 1996).

With the above mentioned data two series of experiment were carried out to answer the following questions: a) does cadmium differentially affect blood distribution of T and B cells? and b) do cadmium effects on B and T cells correlate with the blood cadmium concentrations?

Materials and methods

Animals and experimental design

Experiments were carried out in adult male Sprague-Dawley rats (250–300 g), kept under controlled conditions of light (light between 07.00 and 21.00 h daily) and temperature (22 ± 2 °C). Food and water were available 'ad libitum'. Six groups of 10 animals were used. Five groups were treated for one month with cadmium chloride ($CdCl_2$) at a dose of 5, 10, 25, 50 or 100 ppm of $CdCl_2$ in the drinking water. The other group received water supply from the State Company (the cadmium concentration was of 0.36 μg/L) to use it as control. The doses were selected considering that the rat is more resistant to pollutants than humans. The lower dose of cadmium (as cadmium alone) administered to the animals in this work, is approximately 167 times higher than Provisional Tolerable Weekly Intake (PTWI) of this heavy metal (WHO, 1993). Most of the work done in the literature on cadmium toxicity was reported using the doses of 25 or 50 ppm (Cory-Slechta and Weiss 1981; Sugawara *et al.* 1981; Lafuente *et al.* 2003a,b,c) and it is why we selected two doses lower than 25 ppm to ascertain if the effects of the metal appeared.

At the end of the treatment, rats were killed by decapitation at 12:00 h to avoid the diurnal distribution of lymphocyte subsets, according with unpublished data of the group. Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 5–7 s. From each animal, two samples of the trunk blood were collected in tubes containing EDTA (60 g/L): one sample is to determine the lymphocytes subset populations and the other-one is to measure cadmium concentration. The blood samples to determine the cadmium level were kept frozen at −20 °C until analyzed.

The studies were conducted in accord with the principles and procedures outlined in the NIH guide for the Care and Use of the Laboratory Animals (National Research Council 1996).

Lymphocyte subsets

The relative size distributions of lymph cells in blood were determined by FACS analysis, as previously described (Esquifino *et al.* 2000). For these studies, we used the following specific monoclonal antibodies from PharMingen (Becton Dickinson, USA): R-Phycoerythrin (R-PE)-conjugated mouse anti-rat CD45RA for B lymphocytes (clone OX-33); fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat alpha/beta T-cell receptor for T lymphocytes (clone R73); FITC-conjugated mouse anti-rat CD4 (clone OX-35) which recognize a rat T helper cell differentiation antigen and; R-PE-conjugated mouse anti-rat CD8a (clone OX-8) which recognize the reactive antigen expressed on rat T cytotoxic/suppressor cells.

The cells (a sample of 100 μL of complete blood) were incubated with 20 μL of the appropriate primary antibodies for 15 min at room temperature and dark. After that, 2 mL of lysing solution (PharM Lyse™ from PharMingen, Becton Dickinson, USA) were added, and after a second incubation of 8 min (room temperature and dark), the samples were centrifuged at 500 g for 5 min at 4 °C. The obtained pellet was re-suspended with 2.5 mL of PBS solution, and a second centrifugation (500 g for 5 min at 4 °C) was carried out. The cells were re-suspended in 0.5 mL of 1% paraformaldehyde in PBS. The flow cytometer used in this study is a system FACSCalibur (Becton Dickinson, USA).

Cadmium accumulation

Cadmium concentration was determined in the complete blood of individual animals. Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry after microwave digestion (GFAAS) (López-Artíguez *et al.* 1993). The samples were mineralized in a Parr 4780 microwave acid digestion bomb and a Samsung M-745 microwave oven. The mineralization step was performed by treating 0.5 mL of blood with 3.0 mL of ultrapure nitric acid and 1 mL of distilled water. The mineralization was complete after two digestions at 450 W for 2 min, 20 s each. For cadmium determination, an atomic absorption spectrophotometer (Perkin-Elmer, Varian Spectra 250 plus) with Zeeman background correction was used. Accuracy was obtained by calibration against aqueous standards. For the aqueous standards control, we have checked that the absorbance measures correspond with the technical characteristics of the device, allowing a deviation of 5%. That is to say, the RSD (relative standard deviation) is inferior to the 5% for the samples and for the patterns. Every ten samples a reslope has been made. The lowest level of sensitivity was 0.02 mg/mL. Samples of the whole experiment were analyzed in the same assay to avoid interassay variations; the intraassay coefficient of variation was 4,2%.

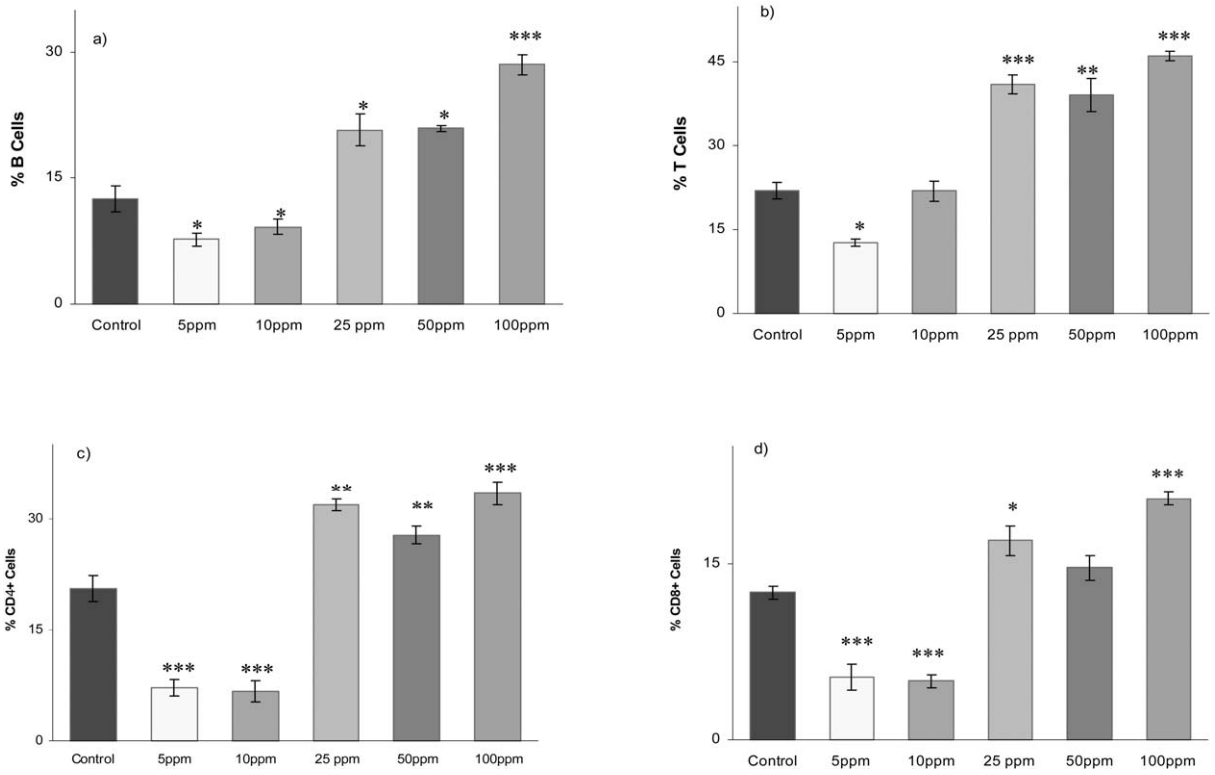

Fig. 1. Effects of cadmium exposure in a) B cells, b) T cells, c) $CD4^+$ cells and d) $CD8^+$ cells percentage in blood, in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

Statistical analysis

Results were analyzed by one way ANOVA followed by a Tukey test for multiple comparations. The results were considered significant at $P \leq 0.05$. All values represent the mean ± S.E.M.

Results

Figure 1 shows the effect of cadmium exposure in blood lymphocyte populations. Cadmium exposure differentially affects the B cell percentage (Figure 1a; $F = 49.29$, $P \leq 0.001$) according with the administered dose of the metal. The lower doses of $CdCl_2$ used (5 and 10 ppm) reduced the percentage of B lymphocytes as compared to the values found in the control group ($P \leq 0.05$ for each comparison, Figure 1a). However, from the dose of 25 ppm of $CdCl_2$ on, the percentage of this population increased as compared to controls ($P \leq 0.05$ for 25 and 50 ppm and $P \leq 0.001$ for the dose of 100 ppm). A similar effect was observed for T lymphocytes (Figure 1b) and its sub-populations $CD4^+$ (Figure 1c) and $CD8^+$ (Figure 1d) cells ($F = 85.82$, $P \leq 0.001$; $F = 64.14$, $P \leq 0.001$; $F = 62.40$, $P \leq 0.001$ respectively), although the reduction in T cells was not statistically significant with the dose of 10 ppm of $CdCl_2$. Also for the subsets of T cells examined, i.e. $CD4^+$ and $CD8^+$cells, a significant depressive effect with the dose of 5 or 10 ppm of $CdCl_2$ was also observed ($P \leq 0.001$ for each comparison) for both sub-populations, as compared to the values found in the control group. From the dose of 25 ppm of the metal on, an increase in the percentage of T cells ($p \leq 0.001$ for 25 and 100 ppm, and $p \leq 0.01$ for the dose of 50 ppm of $CdCl_2$), $CD4^+$ ($P \leq 0.01$ for the doses of 25 and 50 ppm and $P \leq 0.001$ for the dose of 100 ppm of $CdCl_2$), and $CD8^+$ ($P \leq 0.05$ for the dose of 25 ppm and $P \leq 0.001$ for the dose of 100 ppm of $CdCl_2$) cells was also observed, except for the dose of 50 ppm on $CD8^+$cells.

Figure 2 shows blood cadmium concentration. Blood levels of cadmium were differentially affected by cadmium according with the dose used ($F = 25.33$,

$P \leq 0.001$). As expected blood levels of the metal increased as did the dose of the metal considered. The increase was statistically significant for the dose of 10 ppm on ($P \leq 0.05$ for the doses of 10, 25 and 50 ppm of $CdCl_2$ and $P \leq 0.001$ for the dose of 100 ppm of $CdCl_2$).

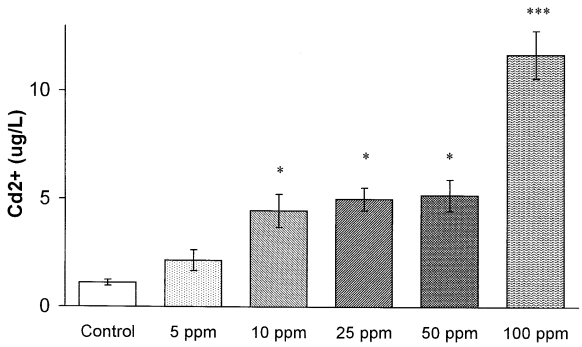

Fig. 2. Cadmium accumulation in blood from adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). $^{*}P \leq 0.05$, $^{***}P \leq 0.001$ vs. control group.

Discussion

Globally, cadmium exposure shows a biphasic effect on the distribution of blood phenotypes: low doses of the metal decreased the percentage, whereas the higher doses of the metal increased the percentages of blood lymphocytes subsets. Novel findings indicate an increase in the circulating percentages of B and T subsets with the higher doses of the metal used. Besides, the results with the lower doses of cadmium used agree with previous data of the literature showing a decreased immune cell response (Descotes 1992).

B cells were decreased in blood with the doses of 5 and 10 ppm. The reduction in the percentage of B cells may be indicative of a reduced humoral response. This effect was previously described using higher doses of the metal than the highest dose used in this study (Gaworski & Sharma 1978, Descotes 1992, Dan *et al.* 2000). Also *in vitro* exposure to high doses of the metal decreased the activity of B cells in humans and in mice (Fujimaki 1987, Steffensen *et al.* 1994). If the reduction in B cells observed after cadmium exposure with the lower doses of the metal is mediated by the stimulation of apoptosis, deserves further investigation, this parameter being stimulated by cadmium (Fujimaki *et al.* 2000). The effects of cadmium on blood B lymphocytes seemed not to be dependent on the blood metal concentration, as cadmium concentration in blood was similar up to the dose of 50 ppm used. Also, an effect of the metal on lymphocyte traffic cannot be discarded (Lafuente *et al.* 2003c).

The changes in T cells are explained by the modifications in the percentages of $CD4^+$ and $CD8^+$ cells observed with any of the dose of the metal used. The increase in the circulating T sub-populations with the higher doses of the metal used may indicate a higher disposability of these cells within the tissues, to react against exogenous stimuli. As indicated above for B lymphocytes, the reduction in T, $CD4^+$ and $CD8^+$ cells observed after cadmium exposure with the lower doses, is mediated by the stimulation of apoptosis, deserves further investigation (Fujimaki *et al.* 2000). As happened for B lymphocytes, the effects of cadmium on blood T lymphocytes seemed not to be dependent on the metal concentrations, as differential effects, according with the dose of cadmium considered, were observed whereas the concentration of the metal reached in blood is similar with any dose up to 50 ppm with the exemption of the highest dose of the metal. Also, as happened for B lymphocytes an effect of the metal on lymphocyte traffic cannot be discarded (Lafuente *et al.* 2003c).

Indirect effects of the metal, which were not explored in this work, may be responsible of the observed changes. Among them changes in the activity of the autonomic nervous system activity may explain at least in part such effects, as in previous studies of the group analyzing the modulatory capacity of this system on the inflammatory reaction (Cardinali *et al.* 1998; Lafuente et al., 2001). Also changes in the activity of the hypothalamic-pituitary-adrenal axis may account for the differences observed, as acute administration of the metal reduced plasma levels and pulsatility of ACTH (Lafuente & Esquifino 1998) and sub-chronic administration of cadmium resulted in an increased plasma ACTH levels (Lafuente *et al.* 2000). Other pituitary hormones like prolactin that is also modified by cadmium exposure (Lafuente *et al.* 2000) can also mediate its effects on B cells as in previous studies using other experimental approaches (Arce *et al.* 1997).

The effect of the metal on blood lymphocyte subsets are tissue specific as cadmium differentially affects the lymphocyte distribution on other immune tissue such as the thymus or the spleen (Lafuente *et al.* 2003c).

In conclusion, the results on the distribution of blood lymphocyte subsets suggest that cadmium inhibits the humoral and cellular immune response with the lower doses of the metal used, but opposite effect are demonstrated with higher doses of cadmium, these effects not being related to circulating cadmium.

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