Toxicology Letters 256 (2016) 33–43

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

# Strain differences of cadmium-induced toxicity in rats: Insight from spleen and lung immune responses

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## HIGHLIGHTS

- Strain differences in acute cadmium immunotoxicity are explored in DA and AO rats.
- Cadmium affected spleen and lungs cells immune response in DA and AO rats.
- The degree of some changes differ between strains in both compartments.
- Data depict complex influence of genetic background on the cadmium immunotoxicity.

## ARTICLE INFO

*Article history:*
Received 19 February 2016
Received in revised form 16 May 2016
Accepted 20 May 2016
Available online 24 May 2016

*Keywords:*
Cadmium
Rats
Albino Oxford (AO) and Dark Agouti (DA) strains
Spleen
Lungs

## ABSTRACT

The impact of genetic background on effects of acute *i.p.* cadmium administration (0.5 mg/kg and 1 mg/kg) on basic immune activity of spleen and lungs was examined in two rat strains, Albino Oxford (AO) and Dark Agouti (DA), known to react differently to chemicals. More pronounced inhibition of Concanavalin A (ConA)-induced and Interleukin (IL)-2 stimulated spleen cell proliferation as well as higher levels of nitric oxide (known to decrease cell's proliferative ability) in DA rats at 1 mg/kg, along with greater inhibition of ConA-induced Interferon (IFN-γ)-production by total and mononuclear (MNC) spleen cells and IL-17 production by spleen MNC in DA *vs.* AO rats at this dose show greater susceptibility of this strain to Cd effects on spleen cells response. More pronounced infiltration of neutrophils/CD11b$^+$ cells to lungs of DA rats treated with 1 mg/kg of Cd and decreased IL-17 lung cell responses noted solely in DA rats speaks in favor of their higher susceptibility to this metal. However, lack of strain disparity in lung cells IFN-γ responses show that there are regional differences as well. Novel data from this study depict complexity of the influence of genetic background on the effects of cadmium on host immune reactivity.



## 1. Introduction

Cadmium (Cd), an industrial pollutant and component of cigarette smoke, is one of the most toxic metals in the environment (Morselt, 1991). It adversely affects a number of organs and tissues such as liver and kidneys (which are cadmium prime targets), as well as lungs, testes and brain (U.S. Department of Health and Human Services, 1997; WHO, 1992). Effects of cadmium on the components of immune system were less investigated and showed both suppression (Marth et al., 2001; Pathak and Khandelwal, 2007) and stimulation (Olszowski et al., 2012).

The susceptibility of tissues to cadmium toxicity is associated with several host physiological parameters including age and sex (Gochfeld, 1997). Differences in cadmium hepatotoxicity were observed between young and adult mice (Shaikh et al., 1993) and rats (Yamano et al., 1998) and in hepatotoxicity and nephrotoxicity

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http://dx.doi.org/10.1016/j.toxlet.2016.05.022
0378-4274/

between male and female rats (Gubrelay et al., 2004). Host genetic variability was considered as a factor that could identify the degree of susceptibility to metals in general (Gochfeld, 1997), but the factors that render some animals more prone to toxicity than the others are not well known. Strain differences noted in hepatotoxicity and testicular toxicity of cadmium in rats were shown as related to the levels of its accumulation in liver (Shaikh et al., 1993; Shimada et al., 2004) and testes (Shimada et al., 2009, 2011). However, several studies showed that cadmium burden is less important than host genetic background, as strain differences in hepatotoxicity (Kuester et al., 2002) and testicular toxicity (King et al., 1998; Liu et al., 2001) were noted in the presence of similar tissue concentrations of this metal. Differential susceptibility of some tissues to cadmium has been ascribed to differences in the activity of these tissues cells. Kupffer cells (liver macrophages) were shown to be the major determinant of differential susceptibility of Fischer 344 and Sprague-Dawley rats (which accumulated similar levels of cadmium in liver) to hepatotoxicity of cadmium (Kuester et al., 2002). Differential intensity of leukocyte infiltration into lungs accounted for strain differences in the susceptibility of C57BL/6 and DBA mice (which accumulated similar levels of metal) to cadmium-induced pulmonary injury (McKenna et al., 1997). These two studies showed not only the contribution of cells of immune system to strain differences in cadmium tissue toxicity in experimental rodents, but they also suggested the influence of genetic variations to cadmium toxicity to immune system. Indeed, one early study showed differential effects of cadmium administration on thymic lymphocytes of Brown-Norway and Lewis rats, although the same amount of this metal was observed in thymuses of both strains (Morselt et al., 1988). Examination of *in vitro* cadmium exposure on cytokine responses by human peripheral blood mononuclear cells showed high level of inter-individual variability of cytokine mRNA levels (coefficient of variation between distinct blood donors of up to 68%) (Marth et al., 2001). Despite these early data, the impact of genetic background on cadmium toxicity to cells of immune system is largely unknown.

Differential effects of cadmium on components of immune system (suppression, stimulation or lack of the impact) were reported in the literature, most probably due to differences in sources of immune cells (blood, spleen, thymus, lungs) used in the studies. Differential effects of cadmium in the same tissue (spleen) might be ascribed to differences in the activities examined (Demenesku et al., 2014) In view of the above cited findings the aim of the present paper was to examine the impact of genetic background on immunomodulatory potential of cadmium by analyzing its effects on spleen and lung immune responses comparatively in Albino Oxford (AO) and Dark Agouti (DA) rats (strains which are known to differ in immune-mediated tissue responses). To this aim, basic aspects of immune activity of spleen and lung leukocytes were analyzed in animals of these strains following acute intraperitoneal (*i.p.*) cadmium administration. The data obtained showed complexity of the influence of genetic background on the effects of cadmium on host immune reactivity, which is a novel finding.

## 2. Materials and methods

### 2.1. Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Serva, Feinbiochemica (Heidelberg, Germany) and was prepared in sterile pyrogen-free saline (Hemofarm AD, Vršac, Serbia). Concanavalin A (ConA), deoxyribonuclease I (DNase I), *o*-dianisidine dihydrochloride, three-(4,5-dimethyl-thiazol-2-yl)-2,5 diphenyl-tetrazolium bromide (MTT), myeloperoxidase (MPO), N-(1-naphtyl) ethylenediamine dihydrochloride, sulfanilamide (*p*-aminobenzenesulfonamide) were all purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Collagenase (type IV) purchased from Worthington Biochemical Corporation (Lakewood, USA), hydrogen peroxide ($H_2O_2$) from Zorka Farma Sabac (Serbia) and sodium nitrite from Fluka Chemika (Buchs, Switzerland) were used in experiments. Culture medium RPMI-1640 (Biowest, Nuaillé, France) supplemented with 2 mM glutamine, 20 μg/ml gentamycine (Galenika a.d., Zemun, Serbia), 5% (v/v) heat inactivated fetal calf serum (Biowest, Nuaillé, France) was used in cell culture experiments. For use in experiments ConA was dissolved in RPMI-1640 medium. All solutions for cell culture experiments were prepared under sterile conditions and sterile filtered (Minisart, pore size 0.20 μm, Sartorius Stedim Biotech, Goettingen, Germany) before use. $^3$H-thymidine (GE Healthcare, Little Chalfont, UK) was prepared in culture medium. Monoclonal antibody OX-42 (mouse anti-rat CD11b/CD11c) was purchased from AbD Serotec (Serotec Ltd., Oxford, UK). Lysis buffer (used for red blood cell lysis), phycoerythrin (PE) labeled $F_{(ab')2}$ goat anti-mouse IgG and mouse anti-rat CD25 were from eBioscience (eBioscience Inc., San Diego, CA, USA). Recombinant human interleukin-2 (IL-2; *E. coli*-derived, Ala21-Thr153) was obtained from R&D Systems (Minneapolis, USA).

### 2.2. Animals and cadmium treatment

All animal procedures were complied with the Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and was approved by the Ethical Committee of the Institute for Biological Research "Sinisa Stankovic" (IBISS), University of Belgrade, Serbia. Twelve to fourteen weeks-old male Dark Agouti (DA) and Albino Oxford (AO) rats, conventionally housed at IBISS, in controlled environment (21/24 °C temperature, a 60% relative humidity and 12-hour (h) light:dark cycle) were used. All rats had *ad libitum* access to standard rodent chow and filtered water throughout the study.

Four to six animals were assigned per group per experiment in two independent experiments. Sterile filtered $CdCl_2$ was administered by *i.p.* injection at a concentration at which animals received 0.5 or 1 mg of cadmium/kg body weight (b.w.) in a 0.5 ml dosing volume. Control group was administered with pyrogen-free saline solely. All measurements were carried out 48 h post-cadmium exposure in animals anesthetized by *i.p.* injection of 40 mg/kg b.w. of thiopental sodium (Rotexmedica, Tritau, Germany).

### 2.3. Cadmium determination

Atomic absorption spectrometry graphite tube technique (AAS Varian 1275; graphite tube, GTA-95, Palo Alto, CA, USA) was used for cadmium content determination in liver, spleen, kidneys and lungs. Lyophilized tissue samples were homogenized and digested in a microwave digestion system (MBS-9, CEM Innovators, Buckingham, UK) in a mix of concentrated HCl and $HNO_3$ (metal-free), filtered and diluted using metal-free ultrapure water. Reference materials were used as control samples: SeronormTM Trace Elements Serum L-1 and ClinChek Plasma Control. Method limit of detection (LOD) was 0.1 mg/kg (0.00089 mmol/kg) and limit of quantification (LOQ) 0.3 mg/kg (0.00267 mmol/kg). The concentrations were expressed as μmol of Cd per kg of wet tissue.

### 2.4. Clinical biochemistry

Heparinized plasma obtained from whole blood samples collected at necropsy, was assessed for levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity using an autoanalyzer (Ciba Corning Express, Oberlin,