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Cadmium modulates hematopoietic stem and progenitor cells and skews toward myelopoiesis in mice

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ABSTRACT

The heavy metal cadmium (Cd) is known to modulate immunity and cause osteoporosis. However, how Cd influences on hematopoiesis remain largely unknown. Herein, we show that wild-type C57BL/6 (B6) mice exposed to Cd for 3 months had expanded bone marrow (BM) populations of long-term hematopoietic stem cells (LT-HSCs), common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs), while having reduced populations of multipotent progenitors (MPPs) and common lymphoid progenitors (CLPs). A competitive mixed BM transplantation assay indicates that BM from Cd-treated mice had impaired LT-HSC ability to differentiate into mature cells. In accordance with increased myeloid progenitors and decreased lymphoid progenitors, the BM and spleens of Cd-treated mice had more monocytes and/or neutrophils and fewer B cells and T cells. Cd impaired the ability of the non-hematopoietic system to support LT-HSCs, in that lethally irradiated Cd-treated recipients transplanted with normal BM cells had reduced LT-HSCs after the hematopoietic system was fully reconstituted. This is consistent with reduced osteoblasts, a known critical component for HSC niche, observed in Cd-treated mice. Conversely, lethally irradiated control recipients transplanted with BM cells from Cd-treated mice had normal LT-HSC reconstitution. Furthermore, both control mice and Cd-treated mice that received Alendronate, a clinical drug used for treating osteoporosis, had BM increases of LT-HSCs. Thus, the results suggest Cd increase of LT-HSCs is due to effects on HSCs and not on osteoblasts, although, Cd causes osteoblast reduction and impaired niche function for maintaining HSCs. Furthermore, Cd skews HSCs toward myelopoiesis.

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal mainly derived from industrial activities. Humans are exposed to Cd primary through smoking or inhalation of Cd-contamined dusts, occupational exposure, and consumption of Cd-contaminated foods (Nawrot et al., 2010). As have been reported, Cd is detectable in urine and blood for a large number of humans in the world, especially for those who work or worked in battery manufacturers (Bulat et al., 2009; Min and Min, 2016; Palus et al., 2003; Sun et al., 2016). The half-life of Cd in human is estimated to be over 10 years (Amzal et al., 2009). Therefore, identification of adverse effects of Cd on human health is important for public health; a good number of studies on Cd toxicities have been reported (Nawrot et al., 2010; Nordberg, 2009; Thevenod and Lee, 2013).

Among studies of Cd toxicology, one concern is the toxicity of Cd to the immune system (Hanson et al., 2012; Ninkov et al., 2015). However, although Cd can influence immunity, Cd modulations are complex, in that increased and reduced immune cell numbers and enhanced and impaired host immunity have been reported (Demenesku et al., 2014; Hanson et al., 2012; Holaskova et al., 2012; Lafuente et al., 2004; Pathak and Khandelwal, 2008). Alteration of the number of immune cells is probably associated with the change of host resistance to pathogens. Studies that were performed to investigate directly the effects of Cd on immune cell development also have been reported. For instance, exposure to Cd is shown to be capable of modulating T cell development in mice (Hanson et al., 2010; Holaskova et al., 2012; Viau et al., 2007). Apart from the immune system, Cd is documented to cause osteoporosis, which is associated with increased osteoblast apoptosis and imbalance of osteoblasts and osteoclasts (Brzoska and Moniuszko-Jakoniuk, 2004a, 2004b; Jin et al., 2004). Activation of various caspases and mitogen-activated protein kinases pathway has been suggested to contribute to osteoblast death induced by Cd exposure (Brama et al., 2012; Zhao et al., 2015), while initiation of autophagy

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likely has a protective role in preventing Cd-induced cellular death (Liu et al., 2016).

Hematopoiesis is the generation of all blood cells from hematopoietic stem cells (HSCs). Therefore, HSCs have two hallmark properties, namely self-renewal and multipotent differentiation potential. Usually, long-term HSCs (LT-HSCs) give rise to short-term HSCs (ST-HSCs) and the later differentiate into multipotent progenitors (MPPs). MPPs further differentiate into common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). A substantial part of the CMPs give rise to neutrophils and monocytes/macrophages *via* an intermediate state classified as granulocyte-macrophage progenitors (GMPs), while most of the CLPs differentiate into lymphoid cells including B cells, T cells and NK cells (Wang and Wagers, 2011). Currently, specific markers for identification of HSPCs have been developed and broadly implicated (Dutta et al., 2015; Li et al., 2011; McCabe et al., 2015). After birth, most HSCs are located in the bone marrow (BM), where they need to stay in a microenvironment called "niche" to maintain their stemness (Wilson and Trumpp, 2006). As a functional concept, the HSC niche is comprised of multiple types of bone and BM cells, of which the most important cells include osteoblasts and endothelial cells (Morrison and Scadden, 2014). By using gene-targeted mouse models, it has been demonstrated that modifications on osteoblasts or endothelial cells lead to HSC alteration (Ding et al., 2012; Mansour et al., 2012). It is suggested that LT-HSCs mainly reside in the osteoblast niche while ST-HSCs are preferentially located in the endothelial niche (Yin and Li, 2006). Therefore, environmental stresses can influence HSCs by interaction with the HSC itself, HSC niche, or both. For instance, lipopolysaccharide (LPS) is able to activate HSCs by binding to their surface-expressed toll-like receptor 4 (Nagai et al., 2006), while IFNγ interaction on macrophages has been shown to influence HSCs during *Ehrlichia* infection (McCabe et al., 2015).

Although studies have suggested Cd affects hematopoiesis (Hanson et al., 2012), it is still unknown whether Cd modulates hematopoietic stem and progenitor cell (HSPC) differentiation. The aim of this study was to investigate the effects of Cd on hematopoiesis at the HSPC level. Because Cd is known to cause osteoporosis, a process with loss of osteoblasts, we hypothesized that osteoblast apoptosis induced by Cd exposure might lead to impaired maintenance of HSCs in the niche and the consequentially abnormal hematopoiesis. Unexpectedly, we found that exposure to Cd led to an increase of LT-HSCs, albeit less LT potential under competitive condition, using mouse models. On the other hand, the reciprocal BM transplantation assay indicated Cd-treated mice had impaired niche function to support HSCs, which was associated with increased osteoblast apoptosis by Cd exposure. Moreover, we found that Cd promoted myelopoiesis at the expense of lymphopoiesis at the MPP level. Our study reveals a previously unrecognized toxic effect of Cd on HSPCs.

2. Materials and methods

2.1. Mice and Cd treatment

6 to 8-week old wild-type (WT) C57BL/6 (B6) mice and β-actin-driven enhanced green fluorescent protein (GFP) expressing mice with B6 background were purchased from Shanghai SLAC Laboratory Animal Co. LTD and Shanghai Research Center For Model Organisms, China, respectively. Mice were housed in a specific pathogen free animal facility at Fudan University, with a controlled temperature and humidity and 12 h light on/12 h light off. This work was approved by Fudan University Animal Care and Use Committee. Mice were provided *ad libitum* with drinking water with 0 or 10 ppm of $CdCl_2$ for up to 3 months.

2.2. Cd concentration measurement

Blood, BM and bones were harvested from Cd-treated mice and control mice at 3 months post the initiation of treatment. Cd concentration was measured with Atomic Absorption Spectroscopy (AAS) (M6 MK2) (Thermo Fisher Scientific, Waltham, MA) as has been described (Nwokocha et al., 2012).

2.3. Alendronate treatment

WT B6 mice with or without Cd treatment were subcutaneously injected with Alendronate Sodium Trihydrate (Bisphosphonates, CAS: 121268-17-5; TCI, Japan) every other day (50 μg in 200 μL of PBS for each injection) during the entire Cd administration period (Plotkin et al., 1999). Subcutaneous injection of the same volume of PBS was used as vehicle control.

2.4. Flow cytometry

Antibodies (clone) and fluorochromes used in our experiments included: biotin-conjugated anti-Lineage (Lin) panel (B220 (RA3-6B2), CD11b (M1/70), Gr-1 (RB6-8C5), CD3 (145-2C11) and Ter119 (Ter-119)), biotin-conjugated anti-CD45 (30-F11), FITC anti-CD3e (145-2C11), FITC anti-CD11b (M1/70), FITC anti-Gr-1 (RB6-8C5), FITC anti-Ter119 (Ter119), FITC anti-B220 (RA3-6B2), FITC anti-Annexin V, PerCP-Cy5.5 anti-Scal-1 (D7), APC anti-c-Kit (2B8), PE anti-CD34 (HM34), PE-Cy7 anti-CD150 (TC15-12F12.2), PB anti-CD48 (HM48-1), PE-anti-CD48 (HM48-1), PB anti-CD3e (145-2C11), PerCP-Cy5.5 anti-CD16/32 (93), PE-Cy7 anti-CD16/32 (2.4G2), APC anti-CD4 (RM4-5), PerCP-Cy5.5 anti-CD8a (53-6.7), PerCP-Cy5.5 anti-CD11b (M1/70), PE-Cy7 anti-CD19 (6D5), APC anti-Ly6C (HK1.4), PB anti-Ly6C (HK1.4), PE anti-Ly6G (1A8), PE-Cy7 anti-F4/80 (BM8), PE anti-CD51 (RMV-7), APC-Cy7 anti-CD45 (30-F11), APC-Cy7 Streptavidin, PB Streptavidin and 7AAD (Biolegend, San Diego, CA); V500 anti-Scal-1 (D7) and unconjugated anti-CD16/32 (Fc block, 2.4G2) (BD Biosciences, San Jose, CA). Briefly, after erythrocyte lysis with ammonium chloride-based buffer, surface staining was performed by incubation of fresh BM and/or splenic single cell suspensions with antibodies on ice for 30 min in dark. Fc block was used before surface staining if necessary. For osteoblast isolation, femurs and tibias were minced with scissors and then incubated with PBS containing 2 mg/ml of collagenase II and 1× Trypsin (Sigma-Aldrich, St. Louis, MO) with shaking at 37 °C for 30 min (repeated twice) to release osteoblasts (Bakker and Klein-Nulend, 2012). Thereafter osteoblasts were performed surface staining as described (Khurana et al., 2013). Apoptotic cell analysis was performed by incubation with anti-Annexin V in the presence of Annexin V binding buffer at room temperature for 30 min in dark, and 7AAD was used 10 min before reading the cells. With the exception of osteoblasts, BM and splenic cells were fixed with 1% paraformaldehyde in PBS after surface staining. Stained cells were assayed in a BD LSRFortessa, and data were analyzed using FlowJo 9.3.2.

2.5. OP-9 assay

FACS-sorted MPPs (Lin^- c-Kit^{hi}Scal-1^+ $CD48^+$ $CD150^-$) from the BM of control and Cd-treated mice were seeded into wells (500 cells per well) of 24-well plates pre-coated with lethally irradiated (30 Gy) OP-9 cells in α-MEM medium with 10% fetal bovine serum, L-glutamine, sodium bicarbonate, β-mecaptoethanol, GM-CSF, SCF, IL-7, IL-3 and Flt3L (Lai et al., 2005). After culture for 12 days, cells were harvested for analysis for myeloid cells and B cells with a flow cytometry.

2.6. Histology staining of osteoblasts

Femurs and tibias were harvested from control and Cd-treated mice and then fixed with formalin at 4 °C for 48 h. Thereafter, legs were decalcified in the presence of formaldehyde and formic acid for 3 weeks. After that, the decalcified legs were performed regular haematoxylin and eosin (HE) staining.