Ecotoxicology and Environmental Safety 145 (2017) 615–622

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Alteration of the number and percentage of innate immune cells in preschool children from an e-waste recycling area

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ARTICLE INFO

Keywords:
Pb
Cd
E-waste
Innate immune cell
Children

ABSTRACT

Heavy metal lead (Pb) and cadmium (Cd) are widespread environmental contaminants and exert detrimental effects on the immune system. We evaluated the association between Pb/Cd exposures and innate immune cells in children from an electronic waste (e-waste) recycling area. A total number of 294 preschool children were recruited, including 153 children from Guiyu (e-waste exposed group), and 141 from Haojiang (reference group). Pb and Cd levels in peripheral blood were measured by graphite furnace atomic absorption spectrophotometer, NK cell percentages were detected by flow cytometer, and other innate immune cells including monocytes, eosinophils, neutrophils and basophils were immediately measured by automated hematology analyzer. Results showed children in Guiyu had significantly higher Pb and Cd levels than in reference group. Absolute counts of monocytes, eosinophils, neutrophils and basophils, as well as percentages of eosinophils and neutrophils were significantly higher in the Guiyu group. In contrast, NK cell percentages were significantly lower in Guiyu group. Pb elicited significant escalation in counts of monocytes, eosinophils and basophils, as well as percentages of monocytes, but decline in percentages of neutrophils in different quintiles with respect to the first quintile of Pb concentrations. Cd induced significant increase in counts and percentages of neutrophils in the highest quintile compared with the first quintile of Cd concentrations. We concluded alteration of the number and percentage of innate immune cells are linked to higher levels of Pb and Cd, which indicates Pb and Cd exposures might affect the innate and adaptive immune response in Guiyu children.

1. Introduction

Informal and uncontrolled electronic waste (e-waste) recycling often results in human exposure to harmful chemical contaminants (Heacock et al., 2016). Guiyu, a typical e-waste destination and recycling area in southern China, with nearly a 30-year history of unregulated e-waste disposal, has been reported massive amounts of environmental toxicants, including heavy metals and organic pollutants, in environmental and human samples (Huo et al., 2007; Wu et al., 2010; Xu et al., 2015b; Lu et al., 2016, 2017; Zhang et al., 2016). Our previous studies showed that higher lead (Pb) and cadmium (Cd) levels are present in placenta, umbilical cord blood, peripheral blood and urine in the Guiyu population (Zheng et al., 2008; Guo et al., 2010; Xu et al., 2016; Zeng et al., 2016, 2017).

Heavy metal Pb and Cd are widespread environmental contaminants, which cause extensive concern for their adverse effects on health (Jorissen et al., 2013; Dudka et al., 2014). Pb is toxic to the central nervous, hematopoietic, renal, and immune systems (Garcia-Leston et al., 2012; Cabral et al., 2015; Salamat et al., 2017). Previous studies found that Pb exposure affects the humoral and innate immune responses, lymphocyte function and cytokine production (Dyatlov and Lawrence, 2002; Lawrence and McCabe, 2002). Cd is a highly hazardous metal which causes nephrotoxicity, teratogenicity, neurotoxicity, immunotoxicity and endocrine and reproductive toxicities (Wang and Du, 2013; Rani et al., 2014; Priyadarshani et al., 2015). Cd exposure has also been associated with risk of cardiovascular disease and cancer (Maret and Moulis, 2013; Vilahur et al., 2015). In recent years, Pb and Cd immunotoxicity on humoral and cell-mediated immunity

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http://dx.doi.org/10.1016/j.ecoenv.2017.07.059
Received 14 March 2017; Received in revised form 26 July 2017; Accepted 26 July 2017

have been well documented, as mentioned above, but few reports have characterized their impact on innate immunity.

Innate immune cells, including macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer (NK) cells and NKT cells, provide the first line of defense against pathogens and viral infections through recognition of pathogen-associated molecular patterns, via a limited number of germline-encoded pattern recognition receptors, and secretion of a series of cytokines and chemokines to eliminate pathogens and facilitate the adaptive response (Martin, 2014; Ward and Rosenthal, 2014; Iwasaki and Medzhitov, 2015). A handful of studies demonstrate that Pb exposure decreases host resistance to pathogens and viral infections (Nain and Smits, 2011). Cd exposure suppresses specific immune responses while increasing neutrophil activity and macrophage phagocytosis (Sovenyi and Szakolczai, 1993; Demenesku, 2016). With regard to the mechanisms of Pb and Cd toxicity on innate immunity, current evidence suggests that Pb and Cd interact with DNA repair mechanisms, generate reactive oxygen species and induce apoptosis, as well as alter cytokine secretion (Nain and Smits, 2011; Breton et al., 2013; Thevenod and Lee, 2013).

The immune system of preschool children is in the process of maturing, making it particularly sensitive to environmental toxicants (Huo et al., 2007; Xu et al., 2015a; Dai et al., 2017; Lin et al., 2017). To date, there have been no studies reporting the alteration of the innate immune homeostasis affected by heavy metal exposure in Guiyu children. This study aimed to determine the blood Pb and Cd levels of children who reside in Guiyu area, and evaluate toxic effects on innate immune cells, in order to get a deeper understanding of Pb and Cd immunotoxicity on susceptible populations, thereby making an early assessment of the risk for diseases.

2. Materials and methods

2.1. Study population

A total of 294 preschool children, 3–7 years of age, were recruited from Guiyu (n = 153) and Haojiang (n = 141) in December 2011. We selected Haojiang as the reference group, which has similarities with Guiyu in population, cultural background and socioeconomic status, but lacks electronic waste pollution. Questionnaires, including general characteristics of both parents and children, child behavior habits, diet and health physiological parameters, dwelling environments, parent education and jobs, were delivered to the participants and their parents who gave written informed consent prior to enrollment. The study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Sample collection

Whole blood samples were obtained from volunteers, collected in Pb-free tubes by trained nurses, and transported to the laboratory. Blood sample tubes containing EDTA were used for blood routine examination and heavy metal measurement.

2.3. Blood cell examination

Blood cell counts were immediately measured by an automated hematology analyzer (Sysmex XT-1800i, Japan) in a hospital not far from the laboratory. NK cells ($CD3^-CD56^+CD16^+$) were detected by labeled antibodies: MultiTEST CD45/CD3/CD56/CD16 (BD Bioscience, America), and data were collected by an Aria II flow cytometer (BD Bioscience, America) and analyzed with DVIA software (version 6.1, BD Bioscience).

2.4. Pb and Cd measurement

Blood Pb and Cd levels were measured by graphite furnace atomic absorption spectrophotometer (Jena Zeenit 650, Germany), using detection methods according to our previously described publication (Yang et al., 2013). For Pb determination, the main parameters were: a wavelength of 283.3 nm, a lamp current of 4.0 mA, a slit width of 0.8 nm, drying at 90 °C, 105 °C, and 120 °C, ashing at 950 °C, and atomization at 1500 °C. The 0.5% nitric acid solution was used as blank, and the limit of detection (LOD) of this method was 0.51 μg/L (0.051 μg/dL). The accuracy of the method was controlled by recoveries between 95% and 107% from the spiked blood samples. For Cd determination, the main parameters were: a wavelength of 228.8 nm, a lamp current of 4.0 mA, a slit width of 1.2 nm, drying at 90 °C, 105 °C, and 120 °C, ashing at 300 °C, and atomization at 1300 °C. The 2.0% nitric acid solution was used as blank, and the LOD of this method was 0.05 μg/L. The accuracy of detection method was controlled by recoveries between 100% and 103% from the spiked blood samples. Repeated analyses of standard solutions confirmed the method's precision.

2.5. Statistical analysis

Summary statistical analyses were performed using IBM SPSS 19.0 software. Mean ± SD were used to depict blood Pb and Cd concentrations, absolute counts and percentages of monocytes, eosinophils, neutrophils, basophils and NK cells. Chi-square and independent-sample *t*-tests were used to detect differences between the exposed and reference groups for categorical variables and continuous variables, respectively. We adopted a univariate linear regression analysis to analyze the impact of possible relevant factors on Pb and Cd exposure. Separate regression models were used to estimate the association between innate immune cell levels and Pb and Cd exposure, with each exposure categorized by quintiles. All linear models for group differences, in the changes of Pb and Cd concentrations and several innate immune cell levels, controlled for potential confounding variables, such as child gender, age and body mass index (BMI). A $p < 0.05$ in a two-tailed test was determined to be statistically significant.

3. Results

3.1. Characteristics of the study population

Descriptive statistics for the sample characteristics are presented in Table 1. The mean child age in the exposed group was 5.1 years, higher than the 4.4 years in the reference group ($p < 0.01$). In addition, the mean child BMI was significantly lower in the exposed group than that in the reference group (14.73 kg/m^2 versus 16.06 kg/m^2, $p < 0.01$). No significant differences between two groups were found for gender. Differences between the two groups for categorical variables, such as the duration of the child outdoor play, habit of biting pencils and erasers, consumption of dairy and bean products, child e-waste contact, frequency of colds, the duration of residence of both child and parents in the local area, the distance of residence from the road, e-waste contamination within 50 m of the residence, and use of the residence as an e-waste recycling workplace, were all significant different (all $p < 0.05$).

3.2. Candidate factors associated with Pb and Cd levels

Pb and Cd both showed higher mean concentrations in the exposed group (10.34 ± 4.75 μg/dL and 2.39 ± 1.16 μg/L, respectively) than the reference group (8.30 ± 3.01 μg/dL and 1.79 ± 0.45 μg/L, respectively) (Fig. 1). A subsequent univariate linear regression model was used to determine the relationship between possible factors and two heavy metal exposures (Table 2). We took into account the child's age, BMI and gender as potential covariates in the change of Pb and Cd concentrations. Therefore, these variables were being controlled. We found child blood Pb levels were positively associated with the