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
Available online 12 August 2017
0147-6513/

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

Table 1
Descriptive statistics for the study population.

Characteristics	Exposed group (n = 153)	Reference group (n = 141)	*p*
Child age (years)	5.1 ± 1.2	4.4 ± 0.9	< 0.001[a]
Gender [n (%)]	153	141	0.930[b]
male	93 (60.8)	85 (60.3)	
female	60 (39.2)	56 (39.7)	
Child body mass index (BMI, kg/m^2)	14.73 ± 1.17	16.06 ± 1.41	< 0.001[a]
Duration of the child outdoor play (h) [n (%)]			0.003[b]
~0.5	26 (17.2)	6 (4.7)	
~1.0	51 (33.8)	40 (31.0)	
~2.0	53 (35.1)	48 (37.2)	
~3.0	16 (10.6)	27 (20.9)	
> 3.0	5 (3.3)	8 (6.2)	
Habit of biting pencils and erasers [n (%)]			0.007[b]
none	102 (67.5)	106 (79.7)	
occasionally	41 (27.2)	27 (20.3)	
frequently	8 (5.3)	0 (0)	
Dairy product consumption frequency [n (%)]			0.002[b]
none	12 (7.9)	3 (2.2)	
1–3 times/month	36 (23.9)	33 (25.0)	
1–3 times/week	74 (49.0)	48 (36.4)	
daily	29 (19.2)	48 (36.4)	
Bean product consumption frequency [n (%)]			0.011[b]
none	9 (5.9)	3 (2.4)	
1–3 times/month	93 (61.2)	64 (50.4)	
1–3 times/week	43 (28.3)	58 (45.7)	
daily	7 (4.6)	2 (1.5)	
Residence as e-waste recycling workplace [n (%)]			< 0.001[b]
no	62 (41.1)	119 (89.5)	
yes	89 (58.9)	14 (10.5)	
Residence duration of child in local area (years) [n (%)]			< 0.001[b]
~ 1	10 (6.5)	13 (9.2)	
~ 3	15 (9.8)	39 (27.6)	
~ 6	128 (83.7)	89 (63.2)	
Residence duration of father in local area (years) [n (%)]			< 0.001[b]
~ 1	2 (1.4)	3 (2.3)	
~ 5	11 (7.4)	10 (7.6)	
~ 10	9 (6.1)	31 (23.7)	
> 10	126 (85.1)	87 (66.4)	
Residence duration of mother in local area (years) [n (%)]			< 0.001[b]
~ 1	4 (2.7)	10 (7.6)	
~ 5	11 (7.4)	38 (28.8)	
~ 10	26 (17.4)	40 (30.3)	
> 10	108 (72.5)	44 (33.3)	
Distance of residence from road (m) [n (%)]			< 0.001[b]
< 10	59 (39.0)	19 (14.6)	
~ 50	40 (26.5)	24 (18.5)	
~ 100	27 (17.9)	33 (25.4)	
> 100	25 (16.6)	54 (41.5)	
E-waste contamination within 50 m away from house [n (%)]			< 0.001[b]
no	35 (23.2)	131 (96.3)	
yes	116 (76.8)	5 (3.7)	
Child e-waste contact [n (%)]			< 0.001[b]
no	87 (59.6)	120 (93.8)	
yes	59 (40.4)	8 (6.2)	
Child vitamin and calcium intake in the past year [n (%)]			0.008[b]
none	22 (14.6)	31 (24.1)	
rarely	69 (45.7)	68 (52.7)	
occasionally	53 (35.1)	23 (17.8)	
frequently	7 (4.6)	7 (5.4)	
Child cold frequency in the past year [n (%)]			0.013[b]
0–3	74 (48.7)	54 (40.9)	
4–6	37 (24.3)	52 (39.4)	
7–9	23 (15.1)	20 (15.2)	
> 10	18 (11.9)	6 (4.5)	

[a] Analysis by independent-sample *t*-test.
[b] Analysis by chi-square test.

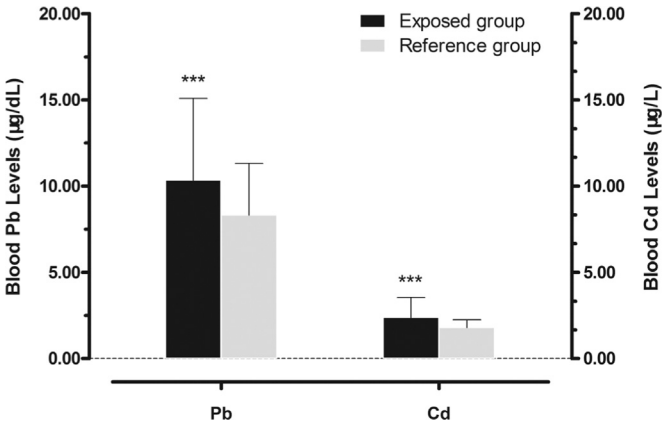

Fig. 1. Pb and Cd levels in peripheral blood of children. Exposed group, n = 153; Reference group, n = 141. Results are presented as mean ± SD; analysis by independent-sample *t*-test. Value of ***$p < 0.001$ were considered statistically significant.

residence duration of child in the local area ($\beta_{unadjusted}$ = 0.845, 95% CI: 0.076, 1.615), the residence duration of father in the local area ($\beta_{unadjusted}$ = 1.087, 95% CI: 0.383, 1.791), the residence duration of mother in the local area ($\beta_{unadjusted}$ = 1.070, 95% CI: 0.544, 1.595), the residence as an e-waste recycling workplace ($\beta_{unadjusted}$ = 1.346, 95% CI: 0.332, 2.361), and the child e-waste contact ($\beta_{unadjusted}$ = 2.636, 95% CI: 1.492, 3.780). However, in the adjusted linear regression model, child blood Pb levels had no correlation with the residence duration of child in the local area ($\beta_{adjusted}$ = 0.622, 95% CI: −0.160, 1.404). In addition, some factors negatively correlated with Pb levels, such as dairy product consumption frequency ($\beta_{adjusted}$ = −0.621, 95% CI: −1.201, −0.041) and distance of residence from the road ($\beta_{adjusted}$ = −0.521, 95% CI: −0.942, −0.100).

In terms of factors associated with blood Cd levels, we found positive correlations between blood Cd levels and certain variables, such as use of residence as an e-waste recycling workplace ($\beta_{adjusted}$ = 0.248, 95% CI: 0.023, 0.474), and the residence duration of child in the local area ($\beta_{adjusted}$ = 0.183, 95% CI: 0.016, 0.350) and the residence duration of mother in the local area ($\beta_{adjusted}$ = 0.136, 95% CI: 0.019, 0.253). Other variables, such as the duration of the child outdoor play ($\beta_{adjusted}$ = −0.140, 95% CI: −0.245, −0.036) and distance of residence from road ($\beta_{adjusted}$ = −0.141, 95% CI: −0.231, −0.052) exhibited negative association with blood Cd levels.

3.3. Distribution of innate immune cells

A general description of several innate immune cell levels is presented in Fig. 2 (A - I). Monocytes, eosinophils, neutrophils, basophils and NK cells were estimated in our study, and expressed as percentage and absolute value. There were higher mean absolute values of monocytes, eosinophils, neutrophils and basophils in the exposed group (0.61×10^9/L, 0.29×10^9/L, 4.24×10^9/L and 0.05×10^9/L, respectively) compared with the reference group (0.51×10^9/L, 0.19×10^9/L, 3.30×10^9/L and 0.04×10^9/L, respectively). Furthermore, there were higher eosinophil and neutrophil percentages in white blood

Table 2
Univariate linear regression analysis of factors related to blood Pb and Cd levels in children.

	Blood Pb levels		Blood Cd levels	
	Unadjusted β (95% CI)	Adjusted β (95% CI)[a]	Unadjusted β (95% CI)	Adjusted β (95% CI)[a]
Duration of the child outdoor play	−0.404 (−0.896, 0.088)	−0.143 (−0.901, 0.074)	−0.151 (−0.255, −0.047)**	−0.140 (−0.245, −0.036)**
Habit of biting pencils and erasers	0.158 (−0.802, 1.118)	0.043 (−0.902, 0.988)	0.063 (−0.142, 0.267)	0.035 (−0.167, 0.237)
Dairy product consumption frequency	−0.709 (−1.291, −0.127)*	−0.621 (−1.201, −0.041)*	−0.044 (−0.170, 0.081)	−0.014 (−0.139, 0.111)
Bean product consumption frequency	0.156 (−0.654, 0.965)	0.165 (−0.631, 0.961)	−0.144 (−0.316, 0.029)	−0.140 (−0.310, 0.029)
Residence as an e-waste recycling workplace	1.346 (0.332, 2.361)**	1.227 (0.176, 2.279)*	0.316 (0.100, 0.532)**	0.248 (0.023, 0.474)*
Residence duration of child in local area	0.845 (0.076, 1.615)*	0.622 (−0.160, 1.404)	0.244 (0.081, 0.407)**	0.183 (0.016, 0.350)*
Residence duration of father in local area	1.087 (0.383, 1.791)**	0.894 (0.185, 1.602)*	0.102 (−0.047, 0.252)	0.053 (−0.098, 0.240)
Residence duration of mother in local area	1.070 (0.544, 1.595)**	0.933 (0.393, 1.473)**	0.182 (0.068, 0.295)**	0.136 (0.019, 0.253)*
Distance of residence from the road	−0.642 (−1.061, −0.224)**	−0.521 (−0.942, −0.100)*	−0.166 (−0.255, −0.078)**	−0.141 (−0.231, −0.052)**
E-waste contamination within 50 m away from house	0.470 (−0.615, 1.556)	0.122 (−0.979, 1.222)	0.177 (−0.062, 0.416)	0.089 (−0.155, 0.333)
Child e-waste contact	2.636 (1.492, 3.780)**	2.389 (1.237, 3.540)**	0.211 (−0.043, 0.464)	0.131 (−0.123, 0.385)
Child vitamin and calcium intake in the past year	−0.095 (−0.726, 0.536)	−0.202 (−0.826, 0.421)	0.001 (−0.135, 0.134)	−0.028 (−0.162, 0.106)
Child cold frequency in the past year	0.270 (−0.241, 0.782)	0.250 (−0.260, 0.760)	0.014 (−0.095, 0.124)	0.019 (−0.091, 0.128)

[a] Model was adjusted by child age, gender and BMI. β : regression coefficient ; CI : confidence interval. Values of $^*p < 0.05$, $^{**}p < 0.01$ were considered statistically significant.

cells and lower NK cell percentages among lymphocytes in the exposed group (3.26%, 47.50% and 14.87%, respectively) than those in the reference group (2.57%, 42.67% and 18.01%, respectively).

3.4. Associations between distribution of innate immune cells and heavy metal exposure

To estimate associations between distribution of different innate immune cells and exposures, we categorized Pb and Cd exposures into quintiles for statistical analysis, and adjusted separate regression models by the child's age (Table 3). For blood Pb, the lowest (2.64–6.31 μg/dL), second (6.31–7.69 μg/dL), third (7.69–9.60 μg/dL), fourth (9.60–12.04 μg/dL) and highest (12.04–37.09 μg/dL) quintile had average blood Pb concentrations of 5.10, 7.11, 8.65, 10.54 and 15.50 μg/L, respectively. We found monocyte (β = 0.083; 95% CI: 0.004, 0.162) and basophil (β = 0.013; 95% CI: 0.001, 0.026) counts were both on average higher in the third quintile of Pb concentration, and eosinophil counts (β = 0.078; 95% CI: 0.004, 0.152) in the fourth quintile compared with those in the lowest quintile (all $p < 0.05$). For percentages of monocytes, results showed increments in all quintiles (second quintile β = 0.765; 95% CI: 0.038, 1.491; third quintile β = 0.976; 95% CI: 0.248, 1.704; highest quintile β = 0.766; 95% CI: 0.036, 1.497) except the fourth quintile (β = 0.358; 95% CI: −0.371, 1.088). However, we observed that neutrophil percentages were on average lower in Pb concentrations in the second quintile compared with the lowest quintile (β = −4.153; 95% CI: −7.792, −0.514) ($p < 0.05$). Neither neutrophil counts nor percentages of eosinophils, basophils and NK cells had significant associations with Pb exposure.

For blood Cd, the lowest (0.47–1.45 μg/L), second (1.45–1.69 μg/L), third (1.69–2.10 μg/L), fourth (2.10–2.59 μg/L) and highest (2.59–7.90 μg/L) quintile had an average Cd of 1.25, 1.56, 1.87, 2.36 and 3.51 μg/L, respectively. Concerning the relationship between

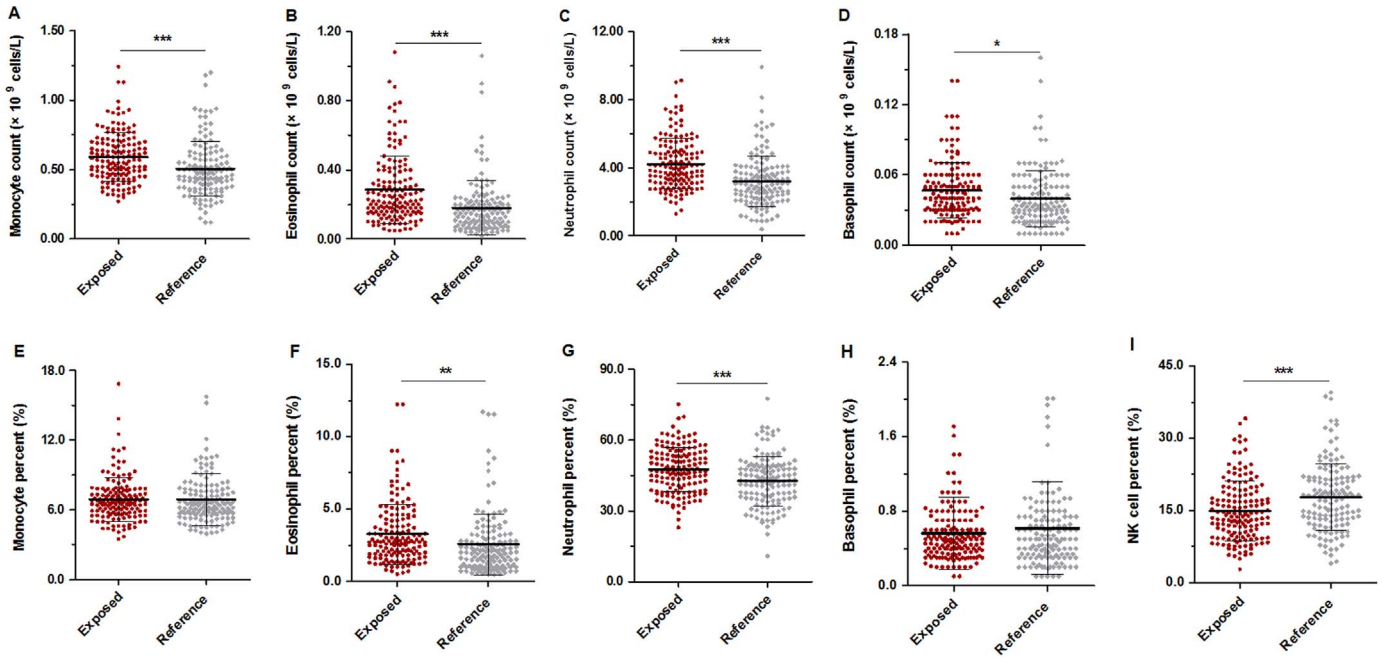

Fig. 2. Innate immune cell counts and percentages in child peripheral blood. A, B, C and D represent innate immune cell counts; E, F, G, H and I show the percentage results between two groups. Exposed group, n = 153; Reference group, n = 141. Results are presented as mean ± SD; analysis by independent-sample *t*-test. Values of $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ were considered statistically significant.

Table 3
Regression coefficients and 95% CIs from linear regression models for innate immune cell levels (10^9/L, %) in children by quintiles of blood Pb (μg/dL) and Cd (μg/L) concentrations.

	n	Mean ± SD	Monocyte count β (95% CI)	Eosinophil count β (95% CI)	Neutrophil count β (95% CI)	Basophil count β (95% CI)	% Monocytes β (95% CI)	% Eosinophils β (95% CI)	% Neutrophils β (95% CI)	% Basophils β (95% CI)	% NK β (95% CI)
Blood Pb (μg/dL)											
2.64–6.31	59	5.10 ± 1.05	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
6.31–7.69	59	7.11 ± 0.36	0.039 (−0.039, 0.118)	0.017 (−0.056, 0.091)	−0.450 (−1.062, 0.162)	0.008 (−0.005, 0.020)	0.765 (0.038, 1.491)*	0.085 (−0.681, 0.851)	−4.153 (−7.792, −0.514)*	0.076 (−0.085, 0.237)	0.301 (−2.211, 2.814)
7.69–9.60	59	8.65 ± 0.53	0.083 (0.004, 0.162)*	−0.015 (−0.089, 0.058)	−0.256 (−0.869, 0.358)	0.013 (0.001, 0.026)*	0.976 (0.248, 1.704)**	−0.247 (−1.015, 0.521)	−1.872 (−5.519, 1.775)	0.105 (−0.056, 0.267)	−0.107 (−2.625, 2.411)
9.60–12.04	59	10.54 ± 0.70	0.053 (−0.026, 0.131)	0.078 (0.004, 0.152)*	−0.052 (−0.667, 0.563)	0.007 (−0.006, 0.020)	0.358 (−0.371, 1.088)	0.657 (−0.113, 1.427)	−2.167 (−5.822, 1.489)	0.033 (−0.129, 0.195)	0.301 (−2.224, 2.825)
12.04–37.09	58	15.50 ± 4.61	0.074 (−0.005, 0.153)	0.009 (−0.065, 0.083)	−0.186 (−0.802, 0.430)	0.002 (−0.011, 0.015)	0.766 (0.036, 1.497)*	−0.024 (−0.795, 0.747)	−2.897 (−6.558, 0.763)	−0.014 (−0.176, 0.148)	0.830 (−1.697, 3.358)
Blood Cd (μg/L)											
0.47–1.45	59	1.25 ± 0.16	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
1.45–1.69	59	1.56 ± 0.07	−0.002 (−0.080, 0.077)	0.004 (−0.070, 0.078)	0.138 (−0.471, 0.748)	−0.004 (−0.016, 0.009)	−0.184 (−0.913, 0.546)	−0.017 (−0.788, 0.753)	1.214 (−2.427, 4.856)	−0.028 (−0.189, 0.134)	1.864 (−0.620, 4.348)
1.69–2.10	59	1.87 ± 0.12	0.052 (−0.026, 0.131)	0.010 (−0.064, 0.084)	0.277 (−0.332, 0.887)	−0.003 (−0.015, 0.010)	0.346 (−0.383, 1.076)	0.070 (−0.700, 0.840)	1.169 (−2.470, 4.808)	−0.003 (−0.165, 0.158)	1.117 (−1.365, 3.600)
2.10–2.59	59	2.36 ± 0.16	−0.015 (−0.094, 0.0163)	0.004 (−0.070, 0.078)	0.266 (−0.344, 0.875)	0.001 (−0.012, 0.013)	−0.481 (−1.211, 0.249)	−0.075 (−0.846, 0.695)	2.518 (−1.124, 6.159)	0.006 (−0.155, 0.168)	0.114 (−2.340, 2.628)
2.59–7.90	58	3.51 ± 1.14	0.068 (−0.011, 0.146)	0.058 (−0.016, 0.133)	0.665 (0.052, 1.278)*	−0.002 (−0.015, 0.011)	0.190 (−0.544, 0.924)	0.494 (−0.281, 1.268)	3.919 (0.259, 7.579)*	−0.077 (−0.239, 0.086)	−1.396 (−3.893, 1.101)

Model was adjusted by age. Values of $^*p < 0.05$, $^{**}p < 0.01$ were considered statistically significant.

innate immune cells and Cd exposure, we only found neutrophil counts (β = 0.665; 95% CI: 0.052, 1.278) increased 0.665 × 10^9/L, and neutrophil percentages (β = 3.919; 95% CI: 0.259, 7.579) increased 3.919% in association with the highest versus the lowest quintile of Cd levels ($p < 0.05$).

4. Discussion

4.1. Higher blood Pb and Cd concentrations in Guiyu children are associated with the candidate factors

Primitive and unregulated e-waste recycling and disposal, such as open incineration and acidic leaching, lead to the emission of heavy metals and persistent organic pollutants, and the accumulation of heavy metals in dust, soil, water, vegetables and paddy rice (Huo et al., 2007; Leung et al., 2008; Xu et al., 2013). Our results are consistent with prior studies demonstrating that soaring Pb and Cd concentrations in Guiyu children are mainly due to the severe pollution of the local environment, as well as the child's habits, diets, contaminant exposure sources and duration of exposure (Zheng et al., 2008; Guo et al., 2010, 2014; Xu et al., 2017). Among all candidate factors investigated, contact of the child with e-waste, parents' dwelling time in the local area and use of residence as an e-waste recycling workplace contribute to the elevated Pb and Cd levels in children. Our previous investigation confirmed that nearly 60–80% of families in Guiyu are engaged in e-waste recycling operations, and most of the recycling workplaces are often residences. These family-run e-waste dismantling activities increase the risk of exposure of children to Pb. Indeed, studies show that the Pb concentration in dust, collected from the workshops, is hundreds of times higher than indoor dust in other areas (Zheng et al., 2008; Srivastava et al., 2011; Guo et al., 2014). We also observed that Pb levels are positively associated with the residence duration of father in the local area, which suggests that fathers with a high risk of Pb exposure can expose children when in close contact. Another possibility is that, because smoking or passive smoking is a source of non-occupational Pb exposure for humans (Liu et al., 2012; Lutz et al., 2012; Behera et al., 2014) and most fathers smoke, smoking could lead to passive smoking by children. Unfortunately, we did not take into account parental smoking as a candidate factor in the study. However, our previous studies have reported parental smoking may promote an increase in child blood Pb and Cd levels (Zheng et al., 2008).

We found factors that can hinder the accumulation of Pb levels, such as the intake of dairy products, which may be protective factors for Pb levels. Other dietary factors, such as bean products and supplements of calcium, iron, zinc and vitamin C, also can be a part of nutritional intervention to reduce Pb absorption (Zheng et al., 2008); however, we are very confused we do not draw such a conclusion. In terms of Cd exposure, our results show that outdoor play time actually negatively correlates with blood Cd levels for children, which is opposite to the early findings (Zheng et al., 2008; Wu et al., 2010). We speculate that indoor exposure to heavy metals may be more serious than outdoor pollution in Guiyu because of the greater density of heavy metal accumulation in dust particles (Argyraki, 2014; Lau et al., 2014).

4.2. Association between Pb and Cd exposures and distribution of innate immune cells in children

We also found blood Pb and Cd levels are associated with altered distribution of innate immune cells in children. Prior studies conducted on immunotoxicity of heavy metals on human leukocytes in vitro suggest that Pb and Cd exposures can interfere with the viability and function of lymphocytes and monocytes (Steffensen et al., 1994; Valentino et al., 2007; Fortier et al., 2008). Our results show that monocyte percentages positively correlate with Pb levels, contrasts a prior study of Pb exposure in male mice (Sharma et al., 2010). Pb and Cd promote inflammation as a consequence of ractive oxygen species

(ROS) production, cell damage and apoptosis, and lead to increases in endogenous waste requiring elimination by monocytes and neutrophils (Pineda-Zavaleta et al., 2004; Cheng et al., 2006; Hebeda et al., 2012). Therefore, it is understandable that Pb exposure might induce increased levels of monocytes.

Strangely, the quintile with blood Pb levels from 6.31 μg/dL to 7.69 μg/dL exhibited a decrease of neutrophil percentages, in contrast to that in the group with Pb levels lower than 6.31 μg/dL. Di Lorenzo et al. has reported the mean absolute neutrophil count is significantly higher in Pb-exposed workers (geometric mean Pb levels: 20.5 μg/dL) with respect to a control group (geometric mean: 3.5 μg/dL) (Di Lorenzo et al., 2006). The different exposure pathways, duration and levels may be reasons for different results. We also observed an upward trend of eosinophil and basophile counts resulting from Pb exposure ($\beta = 0.078$ and $\beta = 0.013$, respectively) ($p < 0.05$). Few prior studies among highly exposed populations have noted positive as well as negative correlations between Pb and eosinophils. In addition to different study populations, the variation in results may also be due to the analyses of divergent adjustment on covariates (Karmaus et al., 2005; Ukaejiofo et al., 2009; Wells et al., 2014).

Our analyses indicate Cd exposure in the highest quintile (2.59–7.90 μg/L) will increase the percentage of neutrophils with respect to the first quintile (0.47–1.45 μg/L), which is similar to a study in rats by Mladenović and colleagues (Mladenovic et al., 2014). However, another study in workers has suggested that individuals with blood Cd levels higher than 1.2 μg/L exhibit a decrease in neutrophil percentages with respect to individuals with Cd levels lower than 1.1 μg/L, is inconsistent with our finding (Ciarrocca et al., 2015). One possible reason for the difference in finding is that our subjects, preschool children, had higher blood Cd levels and lived in the typical e-waste dismantling area. As mentioned above, Cd may elevate monocyte and neutrophil levels via ROS generation, inducing cell apoptosis and increasing endogenous substances. Thus, it is plausible that higher Cd exposure can show a positive association with neutrophil counts and percentages ($\beta = 0.665$ and $\beta = 3.919$, respectively).

From our results, we found decreased percentages of NK cells in Guiyu children. NK cells play critical roles in innate and adaptive immune responses, including cytokine secretion, contact-dependent cell–cell signaling and direct killing of other immune cells, and are involved in combating tumors, viral infections, parasites and bacteria (Van Loo et al., 2010; Holgate, 2012; Deckers et al., 2013; Stringaris et al., 2014). Therefore, lower percentages of NK cells might impair innate immune response, as well as adaptive immune response in Guiyu children. Unfortunately, we can not conclude the effects of Pb and Cd on NK cells, which is similar to previously reported population studies (Yucesoy et al., 1997; Sarasua et al., 2000). However, Sata et al. found a negative correlation between $CD16^+$ NK cells and blood Pb in Pb-exposed workers (Sata et al., 1997). Studies in vitro also have shown that Pb and Cd can inhibit NK cell number and activity (Cifone et al., 1991; Skoczynska et al., 2002).

4.3. Limitations

This study is the first investigation to explore the toxic effect of heavy metals on the innate immune system of preschool children in the e-waste dismantling area, Guiyu. However, there are several limitations of our study. First, we did not analyze some parameters like oxidative stress or innate immunity cytokines to sufficiently conclude that the immunotoxicity of Pb and Cd on innate immune cells. Second, we cannot eliminate the synergistic or antagonistic impact of other environmental pollutants, such as mercury, arsenic, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (BPA), which are widely spread in Guiyu (Guo et al., 2009; Xing et al., 2009; Wu et al., 2011; Ni et al., 2014) and can affect the innate immune response (Miller and Peden, 2014; Thompson et al., 2015). It will be of importance to consider these interactions in our further research. Third, children in the reference group unexpectedly had a mean blood Pb level of 8.30 μg/dL, which exceeds the maximum safe level (< = 5 μg/dL) established by the U.S. CDC (Betts, 2012). Thus we analyzed the association between Pb and innate immune cells by quintiles of Pb concentrations in order to understand the effects of different exposure levels. Other information is required in our questionnaire, including the child's passive smoking and dietary sources, which can enhance the risk of exposure to Pb and Cd (Liu et al., 2012; Lutz et al., 2012; Behera et al., 2014; Guo et al., 2014).

5. Conclusion

Alteration of the numbers and percentages of several innate immune cells is linked to higher levels of Pb and Cd, indicating that Pb and Cd exposures might affect the innate immune response, as well as adaptive immune response in Guiyu children.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21377077, 21577084). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We are grateful to all the recruited children and their guardians for participating in this project.

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