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Exposure to multiple heavy metals associate with aberrant immune homeostasis and inflammatory activation in preschool children

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HIGHLIGHTS

- Higher exposure to Pb, Cd, Hg and As exists in children living in the e-waste area.
- Guiyu group had elevated IL-1 β and IL-6, but lower lymphocyte, IL-1RA and IL-13.
- Neutrophil count was positively correlated with Pb, Cd and Hg exposure.
- Alterations in IL-1 β , IL-6, and IL-1RA relate to exposure to Pb, Cd, Hg and As.

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ABSTRACT

Heavy metals generate adverse health effects by interfering with immune homeostasis and promoting inflammation in individuals. Our objective was to explore the induction of immune and inflammatory responses by multiple heavy metals in children living in the e-waste contaminated area. A total of 147 preschool children were recruited, including 73 children from Guiyu, a typical e-waste recycling area, and 74 from a reference group. Blood levels of heavy metals, including lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As), were detected using an inductively coupled plasma mass spectrometry (ICP-MS). Immune cell counts (neutrophils, monocytes, lymphocytes) were determined by an automatic blood cell analyzer, pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, TNF- α) and anti-inflammatory cytokines (IL-1RA, IL-4, IL-10, IL-13) were analyzed by a Luminex 200 multiplex immunoassay instrument. Multiple correspondences and linear regression analyses were applied to investigate the relationships between heavy metal exposure and relevant parameters. Results shows Guiyu children had higher levels of Pb, Cd, Hg, As, IL-1 β and IL-6, but decreased lymphocyte, IL-1RA and IL-13. Neutrophil count was positively correlated with Pb, Cd and Hg exposure. Anti-inflammatory IL-1RA concentration was negatively related with Pb, Cd, Hg and As, while pro-inflammatory IL-1 β and IL-6 were positively correlated with Pb. Guiyu children may have dysregulated immune response and high inflammation risk. Exposure to Pb, Cd, Hg and As could be harmful for immune response and inflammatory regulation. Our finding of decreased IL-1RA production in children exposed to Pb, Cd, Hg, and As is novel and could be an opportunity for future research.

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1. Introduction

Electronic waste (e-waste) is defined as those discarded electrical and electronic equipment and components, which has become a rapidly increasing global problem (Grant et al., 2013;

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Zeng et al., 2017). E-waste recycling, especially informal and unprotected recycling processes such as acid dissolution, incineration, pyrolysis and gasification, allows the emission of dangerous chemicals, including heavy metals and persistent organic pollutant (Grant et al., 2013). Generally, residents living with close vicinity of an e-waste recycling area are also at risk of environmental exposure due to the contamination in the environment, food and water (Grant et al., 2013). As one of the largest e-waste destinations and recycling areas in China and worldwide, Guiyu has a 30-year history of e-waste disposal and recycling (Chen et al., 2011; Huo et al., 2007). Elevated Pb and Cd levels were found in the placenta, umbilical cord blood, peripheral blood and urine of the Guiyu population (Guo et al., 2010; Zeng et al., 2017; Zheng et al., 2008). Recently, our studies demonstrated that exposure to Pb may contribute to cardiovascular endothelial inflammation and restricted physical growth and development, and cause adverse changes in blood morphology, hemoglobin synthesis and erythrocyte complement receptor 1 expression in Guiyu children (Dai et al., 2017; Zeng et al., 2019; Zheng et al., 2008, 2019).

Growing evidence indicates that heavy metals exert toxicity to individuals by interfering with immune homeostasis and promoting inflammation (Al Bakheet et al., 2013; Brucker et al., 2015; Jin et al., 2016). Heavy metals, such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As), have been sufficiently underscored by prior mechanistic studies. They can cause oxidative stress by inducing the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and promote DNA damage and cell necrosis (Morcillo et al., 2016; Tchounwou et al., 2012; Wang and Shi, 2001). Due to the loss of membrane integrity, necrotic cells release intracellular inflammatory molecules to the extracellular space and consequentially provoke inflammation activation (Bae et al., 2014; Iyer et al., 2009). Epidemiological studies and animal models have indicated that chronic inflammatory diseases, such as type 2 diabetes, gout, recurrent pericarditis, heart failure, rheumatoid arthritis and smoldering myeloma, are strictly related to high exposure to environmental contaminants (Afridi et al., 2013; Arora et al., 2009; Cavaleri, 2015; Monnet-Tschudi et al., 2006; Rowley and Monestier, 2005).

Inflammation is a part of the complex immune response in the body initiated by injurious stimuli, such as damaged cells, pathogens and stimulants. Evidence suggests that inflammation is strictly linked to the genesis of diseases in humans (Ferrero-Miliani et al., 2007). As a protective response, the function of inflammation is to eliminate injury-inducing agents, clear out necrotic cells, tissues damaged from the original insult, and initiate repair processes and restore the physiological function of the tissue or organ affected (Olszowski et al., 2012; Wessling-Resnick, 2010). However, prolonged inflammation may contribute to a wide range of chronic diseases, including cancer, diabetes, and cardiovascular, neurodegenerative and autoimmune diseases (Giovanni et al., 2015; Hamilton, 2008; Schenkein and Loos, 2013; Tseng and Kung, 2012, 2013; Wu et al., 2014). Inflammation involving the innate and adaptive immune systems requires proper coordination of blood vessels, immune cells and molecular mediators in the development and maintenance of inflammation. In the acute inflammatory phase, neutrophils respond to various harmful stimuli and tissue damage. Studies have confirmed that monocytes/macrophages and lymphocytes (T, B and NK cells) constitute the predominant immune cell infiltrates in chronic inflammation (Deng et al., 2005; Goecke et al., 2000; Habtezion, 2015; Ingersoll et al., 2011). As the largest corpuscle of leukocyte, monocytes in the bloodstream migrate to tissue and differentiate into macrophages and dendritic cells, both of which are capable of phagocytosis, antigen presentation and cytokine production involved in the ongoing inflammatory process and immune response (Hausser et al., 1997; Kumar

and Jack, 2006; Ohradanova-Repic et al., 2016). In addition, numerous studies have demonstrated that critical cytokines, including pro-inflammatory or anti-inflammatory interleukin (IL)-1 β , IL-6, IL-8, tumor necrosis factor (TNF)- α and anti-inflammatory cytokines IL-1 receptor antagonist (IL-1 RA), IL-4, IL-10 and IL-13, play pivotal roles in mediating inflammatory responses as well as in regulating immune homeostasis (Cho et al., 2017; Li et al., 2015). Consequently, inflammation-related immune cells and cytokines circulating in the bloodstream can be used to monitor inflammation in the body.

Concern about the toxicity of heavy metal exposure from e-waste is increasing. In our previous studies, parameters involved in immune and inflammatory regulation, such as aberrant alteration of cytokines, increased memory T cell, low percentages of natural killer (NK) cells and decreased concentrations of hepatitis B vaccine titers, have been reported to be associated with exposure to trace levels of environmental chemicals (Cao et al., 2018; Xu et al., 2015; Zhang et al., 2016a). Even so, unequivocal evidence of immune and inflammation activation in children suffering from early-childhood exposure to heavy metals remains little. Hence, we carried out this study to explore and reveal the alterations in immune and inflammatory response resulting from heavy metal exposure.

2. Materials and methods

2.1. Recruitment and questionnaire survey of the study population

This analysis used partial data from our earlier study (Xu et al., 2015). A total of 147 preschool children, 3- to 7-years of age, were recruited from Guiyu (23°32'N, 116°34'E, the e-waste-exposed area, $n = 73$) and Haojiang (23°17'N, 116°43'E, the reference area, $n = 74$) in December 2014. Haojiang is about 31.6 km to the east of Guiyu, without any e-waste pollution. The two regions both belong to Shantou, Guangdong province, and has similar characteristics in latitude, demographic characteristics, life-styles, eating habits, and traffic conditions, cultural background and socioeconomic status, as described in our previous study (Zeng et al., 2017). All enrolled participants from both Guiyu and Haojiang in this study were somatically healthy and free from medications, inflammation or disease history within 1 month before sampling. All parents or guardians of participants provided written informed consent prior to enrollment. The questionnaire collected detailed information on general characteristics of parents and children: child age, gender and BMI, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m (m) of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop. The study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Collection and processing of blood samples

Venous blood samples were obtained from participants and collected in vacuum tubes by trained nurses from the First Affiliated Hospital of Shantou University Medical College. Blood samples in EDTA-containing vacuum tubes were used for the detection of heavy metals and inflammation-related cells. Blood without anti-coagulants was centrifuged for 10 min at 855 $\times$ g at room temperature, and the isolated serum supernatant was stored at -80 °C until assayed for inflammatory cytokines.

2.3. Determination of heavy metals

The detection methods of heavy metals (Cd, Hg, Pb and As) were according to Lu et al. and Wu et al. (Lu et al., 2015; Wu et al., 2016). Metal concentrations were detected using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x; Agilent, USA) in the Shenzhen Center for Disease Control and Prevention. A standard solution of heavy metals was obtained from the National Institute of Meteorology of China (NIM, Beijing, China).

2.4. Analysis of immune cells

Venous blood samples collected in EDTA-containing vacuum tubes were immediately sent to the First Affiliated Hospital of Shantou University Medical College after drawing from participants. Counts of neutrophils, monocytes and lymphocytes were determined by an automatic blood cell analyzer (XE-2100, Sysmex, Japan).

2.5. Measurement of immune and inflammation regulation-related cytokines

In our previous study, we described multiple immunoassays of 34 cytokines (Zhang et al., 2016a). Immune and inflammation regulatory cytokines in serum, including four pro-inflammatory [interleukin (IL)-1 β , IL-6, IL-8, tumor necrosis factor (TNF)- α] and four anti-inflammatory cytokines [IL-1 receptor antagonist (IL-1 RA), IL-4, IL-10 and IL-13] were quantified by a ProcartaPlex Human Cytokine & Chemokine Panel 1A (Thermo Fisher Scientific, USA). Concentrations of these cytokines were analyzed by Luminex 200 multiplex immunoassay instrument (Luminex, USA).

2.6. Statistical analysis

All statistical analyses were carried out using R v3.6.3, SPSS 25.0 and GraphPad Prism 5.0 software. Categorical and continuous variables were compared using the chi-square test and *t*-test, respectively. Abnormal distributions, including heavy metal level, cell count and cytokine concentration, were presented as the median, and comparisons between two groups were made by the Mann-Whitney *U* test.

Multiple correspondence analysis was conducted to investigate the pattern of relationships between heavy metal exposure and socioeconomic factors from questionnaires. It can provide a structural organization for the variables and categories in a dimensional space that is useful for identifying patterns in the data and associations between the investigated parameters (Lana et al., 2017). Multiple categories, such as child gender, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop (see Table 1), as well as quintile data of heavy metal levels were all located as a point in a two-dimensional space. These factors reflect the situation in the aspects of children's behavior habits, diet, nutritional status, physical growth and development, dwelling environment as well as parents' care awareness. On the other hand, most of them have been reported to have an inverse association with blood Pb levels. For example, milk consumption may be a part of nutritional intervention to reduce Pb absorption and considered as a protective factor for Pb exposure (Zheng et al., 2008).

Multiple linear regression analysis was used to evaluate the association of heavy metal exposure with immune and

inflammation regulatory cells/cytokines. For this analysis, data of heavy metals, cell counts and cytokines were ln-transformed to normalize the distribution. Potential confounders including child age, gender and BMI were adjusted in the models. Model 1 represents a crude model without any adjustment; model 2 was adjusted for child gender, age and BMI; model 3 was adjusted for child age, gender and BMI, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop. Meanwhile, the interaction effects of Pb, Cd, Hg and As on immune cells and cytokines were assessed by a centering methodology in the multiple linear regression models. For hypothesis testing, a $p < 0.05$ in a two-tailed test was considered statistically significant.

3. Results

3.1. Characteristics of the study population

Study participant characteristics for the exposed and reference groups are provided in Table 1. The mean child's age in the exposed group was 5.0 years and 4.6 years in the reference group ($p = 0.007$). The mean child BMI was not significantly different between the two groups. All categories were significantly different between exposed and reference group with the exception of child gender and smoking by family members.

3.2. Elevated heavy metal levels in Guiyu children

Blood concentrations of heavy metals are shown in Fig. 1. The median values of Pb, Cd, Hg and As in the exposed group (37.23 $\mu\text{g/L}$, 0.33 $\mu\text{g/L}$, 1.46 $\mu\text{g/L}$ and 5.91 $\mu\text{g/L}$, respectively) were significantly higher than those in the reference group (22.74 $\mu\text{g/L}$, 0.23 $\mu\text{g/L}$, 1.10 $\mu\text{g/L}$ and 4.18 $\mu\text{g/L}$, respectively) (all $p < 0.001$). It indicates that the risk of heavy metal exposure in the e-waste-exposed area is more serious than the reference group.

3.3. Impact of contributing factors on heavy metal exposure

Multiple correspondence analysis was performed to determine which socioeconomic factors were linked to changes in heavy metal levels. Data of heavy metals from all participants were processed into different percentiles, which are presented in Table S1 (see Supplementary Material). Some categorical variables did not show significant differences in each sub-group of heavy metals determined by the chi-square test (data not shown) and were excluded in the following analysis. A joint plot of category points for the first and second dimensions showed an association of socioeconomic factors to heavy metal distributions (Fig. 2-A, B, C, and D). For instance, in Fig. 2-A, dimension 1 contributed with 53.2% of the inertia, while dimension 2 contributed 33.7%. The first quartile of Pb was likely to be associated with milk consumption frequency and not being born locally, and the fourth quartile of Pb was probably associated with residence used as a workshop, e-waste pollution existing within 50 m of residence, and a lack of milk consumption. For Cd, the first tertile was associated with a residence as not being a workshop, and the highest tertile was associated with occasional ventilation of the house. In addition, the highest tertile of Hg was associated with a long residence time of the mother (> 10 years), and the highest tertile of As was inclined to be associated with variables such as maternal education level (primary) and occasional child hand washing before meals.

Table 1

Descriptive statistics for the study population (n = 147).

Characteristics	Exposed group (n = 73)	Reference group (n = 74)	P
Child age (years)	5.0 ± 0.8	4.6 ± 0.9	0.007 ^a
Gender [n (%)]	73 (100)	74 (100)	0.509 ^b
Male	38 (52.1)	43 (53.1)	
Female	35 (47.9)	31 (41.9)	
Child body mass index (BMI, kg/m ²)	14.97 ± 1.06	15.09 ± 1.99	0.654 ^a
Washing hands before meals [n (%)]	63 (100)	72 (100)	<0.001 ^b
Sometimes	37 (58.7)	15 (20.8)	
Often	21 (33.3)	37 (51.4)	
Always	5 (8.0)	20 (27.8)	
Child milk consumption frequency [n (%)]	68 (100)	72 (100)	0.002 ^b
Never	3 (4.4)	1 (1.4)	
1–3 times per month	25 (36.8)	10 (13.9)	
1–3 times per week	30 (44.1)	35 (48.6)	
everyday	10 (14.7)	26 (36.1)	
Child born locally [n (%)]	68 (100)	72 (100)	<0.001 ^b
No	7 (10.3)	33 (45.8)	
Yes	61 (89.7)	39 (54.2)	
Child e-waste contact [n (%)]	63 (100)	71 (100)	<0.001 ^b
never	47 (74.6)	69 (97.2)	
occasionally	9 (14.3)	2 (2.8)	
e-waste in residence	7 (11.1)	0 (0)	
Family member daily smoking [n (%)]	65 (100)	72 (100)	0.090 ^b
non-smoking	15 (23.1)	32 (44.4)	
~2 cigarettes	14 (21.5)	10 (13.9)	
~10 cigarettes	16 (24.6)	17 (23.6)	
~20 cigarettes	13 (20.0)	8 (11.1)	
> 20 cigarettes	7 (10.8)	5 (7.0)	
Residence used as workshop [n (%)]	63 (100)	71 (100)	<0.001 ^b
No	34 (54.0)	69 (97.2)	
Yes	29 (46.0)	2 (2.8)	
E-waste pollution within 50 m of residence [n (%)]	60 (100)	72 (100)	<0.001 ^b
No	33 (55.0)	69 (95.8)	
Yes	27 (45.0)	3 (4.2)	
Characteristics	Exposed group (n = 73)	Reference group (n = 74)	p
Paternal education level [n (%)]	68 (100)	72 (100)	<0.001 ^b
Primary	7 (10.3)	1 (1.4)	
Junior	38 (55.9)	10 (13.9)	
technical secondary	7 (10.3)	15 (20.8)	
Senior	6 (8.8)	9 (12.5)	
college	10 (14.7)	37 (51.4)	
Maternal education level [n (%)]	67 (100)	72 (100)	<0.001 ^b
primary	9 (13.4)	3 (4.2)	
junior	39 (58.2)	16 (22.2)	
technical secondary	12 (17.9)	11 (15.3)	
senior	3 (4.5)	15 (20.8)	
college	4 (6.0)	27 (37.5)	
Paternal residence time [n (%)]	65 (100)	72 (100)	0.001 ^b
< 1 year	0 (0)	0 (0)	
~5 years	5 (7.7)	3 (4.2)	
~10 years	3 (4.6)	21 (29.2)	
> 10 years	57 (87.7)	48 (66.7)	
Maternal residence time [n (%)]	65 (100)	72 (100)	<0.001 ^b
< 1 year	1 (1.5)	1 (1.4)	
~5 years	5 (7.7)	19 (26.4)	
~10 years	16 (24.6)	31 (43.1)	
> 10 years	43 (66.2)	21 (29.1)	
Distance to road from residence (m) [n (%)]	66 (100)	72 (100)	<0.001 ^b
< 10	24 (36.4)	5 (6.9)	
~50	20 (30.3)	19 (26.4)	
~100	15 (22.7)	9 (12.5)	
> 100	7 (10.6)	39 (54.2)	
Ventilation of house [n (%)]	68 (100)	72 (100)	0.015 ^b
always	60 (88.2)	71 (98.6)	
occasionally	8 (11.8)	1 (1.4)	
Maternal occupation associated with e-waste dismantling [n (%)]	66 (100)	72 (100)	<0.001 ^b
no	44 (66.7)	72 (100)	
yes	22 (33.3)	0 (0)	

^a Analysis by the independent-sample *t*-test.^b Analysis by the chi-square test. Values of *p* < 0.05 were considered statistically significant.

Fig. 1. Level of heavy metals (µg/L) in child peripheral blood. A. Blood Pb levels; B. Blood Cd levels; C. Blood Hg levels; D. Blood As levels. Exposed group: $n = 73$; Reference group: $n = 74$. Data are presented as medians, 25th percentile and 75th percentile. Analysis was by the Mann–Whitney U test. Values of $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ were considered statistically significant.

3.4. Alterations in immune cells and inflammatory cytokines

The median lymphocyte count was lower in the exposed group than in the reference group (3.71×10^9 cells/L versus 4.17×10^9 cells/L) ($p = 0.007$); whereas there were no significant differences in neutrophil and monocyte counts between the two groups (Fig. 3). The median concentration of IL-1 β and IL-6 were higher in the exposed group (0.88 pg/mL and 6.12 pg/mL, respectively) compared with those in the reference group (0.67 pg/mL and 4.51 pg/mL, respectively) (both $p < 0.05$) (Fig. 4). However, anti-inflammatory cytokines IL-1RA and IL-13 were both lower in the exposed group (83.72 pg/mL and 3.67 pg/mL, respectively) compared with those in the reference group (162.21 pg/mL and 4.41 pg/mL, respectively) (both $p < 0.001$) (Fig. 4). The concentrations of IL-8, TNF- α , IL-4 and IL-10 did not differ significantly between the two groups.

3.5. Association between heavy metals and immune cells

Ln-transformed data supported the use of multiple linear regression models for the association of heavy metals and immune cells (Table 2). In the unadjusted model 1, neutrophil count was positively related with Pb, Cd and Hg exposure [β (95% confidence intervals or CI): 0.154 (0.004, 0.304), 0.109 (0.019, 0.237) and 0.157 (0.020, 0.293), respectively]. In model 2 neutrophil count was associated with Pb and Hg [β (95% CI): 0.153 (0.006, 0.309) and 0.177 (0.040, 0.313), respectively]. While in model 3, their relationships still existed [Pb: 0.196 (0.003, 0.394), Hg: 0.157 (0.002, 0.313)]. In addition, Cd also exhibited a positive association with neutrophil count [β (95%): 0.117 (0.028, 0.263)] ($p < 0.05$). Meanwhile, the positive association of monocyte level with Cd exposure [β (95%): 0.109 (0.009, 0.226)] was found in model 3 ($p < 0.05$). The interaction effects of 4 metals on immune cells was also analyzed, however, we did not discover a significant interaction between them (Table S2 in Supplementary Materials).

3.6. Association between heavy metals and immune and inflammation regulatory cytokines

In the linear regression analysis (Table 3), the association between heavy metals and cytokines involved in immune and inflammation regulatory processes were explored. Both Pb and Cd positively correlated with IL-1 β production in the crude model 1 [β

(95% CI): 0.225 (0.002, 0.447) and 0.172 (0.013, 0.357), respectively]; however, only Cd remained a positive relationship with IL-1 β level after adjusting for child age, gender and BMI [β (95% CI): 0.165 (0.023, 0.352)] ($p < 0.05$). Results showed that the association between Pb and IL-6 maintained positive in all 3 models [model 1: 0.287 (0.056, 0.518), model 2: 0.264 (0.038, 0.491) and model 3: 0.327 (0.036, 0.617), respectively] (all $p < 0.05$). The association between heavy metals and anti-inflammatory cytokines (Table 4) indicates that all four heavy metals were negatively related to IL-1RA in Model 1 and 2 (all $p < 0.05$). Interaction associations between metals and pro/anti-inflammatory cytokines were not found (Table S3 in Supplementary Materials).

4. Discussion

In this study, children from the e-waste recycling area had higher heavy metal levels (Pb, Cd, Hg and As) but lower lymphocyte counts in peripheral blood. Altered production of cytokines involved in the immune response and inflammatory regulation were observed. Pro-inflammatory IL-1 β and IL-6 were elevated but anti-inflammatory IL-1RA and IL-13 were decreased. These changes might suggest there is an aberrant immune homeostasis and inflammatory activation in children living in the exposed area. Moreover, our results revealed that exposure to Pb, Cd, Hg and As is associated with the alterations in neutrophils, IL-1 β , IL-6 and IL-1RA.

Our data demonstrated the associations between heavy metal exposure and relevant factors in questionnaires (Fig. 2). Although previous studies have performed similar analyses with regard to the association between confounding factors and heavy metal exposure by linear regression analysis or spearman correlation analysis (Xu et al., 2020; Zhang et al., 2016a), this study applied MCA analysis because it is a multivariate exploratory analysis method for visualizing large datasets of categorical variables (Lana et al., 2017). The contributing factors, such as residence used as an e-waste workshop, maternal occupation associated with e-waste dismantling, and e-waste pollution within 50 m of the residence, were related to the highest quartile of Pb levels. In multiple correspondence analysis, education levels of parents were found to be negatively linked to heavy metal As concentration. We speculated that parents with higher educational levels have healthier awareness to prevent their children from e-waste hazards. Some behaviors involved in the prevention of and protection from e-waste

Fig. 2. A joint plot of category points for the first two dimensions showing the association of relevant variables to blood Pb (A), Cd (B), Hg (C), and As (D) levels. Various identified points represent different variables. The relative distance of points in the same quadrant indicates the correlation level between variables.

exposure, such as high frequency of milk consumption, hand washing before meals, and house ventilation, were respectively associated with the lowest blood levels of Pb, As and Cd. Milk consumption frequency may reflect child nutritional status and intervene with Pb accumulation, it thus is considered as a protective factor for Pb exposure to some extent (Zheng et al., 2008). Heavy metals can accumulate on dust particles and other materials in the environment (Lau et al., 2014). Ventilation and handwashing may decrease the contact opportunity of exposure to heavy metals. Furthermore, the maternal residence time in the e-waste area also determines heavy metal exposure to children, especially those who were born in Guiyu. Previous epidemiological studies have confirmed that those mothers with a long-term residence in an e-waste recycling area had relatively high levels of heavy metals in their blood and milk; these toxins can enter the body of the offspring through the placental barrier or breastfeeding process,

increasing child exposure to heavy metals, accordingly (Arbuckle et al., 2016; Guo et al., 2010; Liu et al., 2016; Rzymiski et al., 2015; Silver et al., 2018).

Immune cells and cytokines play an indispensable role in immune function and inflammatory regulation. We observed that different heavy metals exhibit distinct impacts on neutrophil counts. In the early stage of inflammation, neutrophils in the blood are initially recruited by sensing chemoattractant gradient, then traverse the vascular endothelium, and ultimately reach the sites of infection or inflammatory stimuli within minutes (Fournier and Parkos, 2012; Kolaczowska and Kubek, 2013). Thus, sufficient amounts of neutrophils are required for surveilling and eliminating heterologous microbes and irritants. Although no remarkable difference in neutrophil counts between two groups exist, our results showed positive relationships between neutrophil counts and exposure to Pb and Hg in all 3 models (Table 2). It suggests that the

Fig. 3. Counts of immune cells in child peripheral blood. A. Neutrophil counts; B. Monocyte counts; C. Lymphocyte counts. Exposed group: n = 73; Reference group: n = 74. Data are presented as medians, 25th percentile and 75th percentile. Analysis was by the Mann–Whitney *U* test. Values of **p* < 0.05, ***p* < 0.01, ****p* < 0.001 were considered statistically significant.

Fig. 4. Concentration of inflammatory cytokines in child serum. Production of pro-inflammatory cytokines including IL-1 β , IL-6, IL-8 and TNF- α , are shown in A, B, C and D, respectively. Production of anti-inflammatory cytokines including IL-1RA, IL-4, IL-10 and IL-13, are shown in E, F, G and H, respectively. Exposed group: n = 73; Reference group: n = 74. Data are presented as medians, 25th percentile and 75th percentile. Analysis was by the Mann–Whitney *U* test. Values of **p* < 0.05, ***p* < 0.01, ****p* < 0.001 were considered statistically significant. IL: interleukin; TNF: tumor necrosis factor; RA: receptor antagonist.

impact of individuals' characteristics and social-behavioral factors on the association between neutrophil counts and exposure to Pb and Hg is not obvious. Interestingly, a positive association between Cd exposure and neutrophil counts was found in both model 1 and model 3, while it disappeared in model 2, which may due to the

variation of child age, gender and BMI. A previous population study by Di Lorenzo et al. found an increased number of blood neutrophils in lead-exposed workers (Di Lorenzo et al., 2006), which is in accordance with our result. The animal studies concerning Cd toxicity suggested Cd-treated mice had increased monocytes and

Table 2

Associations between heavy metals and immune cells (n = 147).

	Neutrophil			Monocyte			Lymphocyte		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Pb	0.154 (0.004, 0.304)*	0.153 (0.006, 0.309)*	0.196 (0.003, 0.394)*	0.002 (−0.127, 0.130)	0.001 (−0.132, 0.133)	0.019 (−0.144, 0.183)	−0.113 (−0.269, 0.044)	−0.149 (−0.307, 0.008)	−0.045 (−0.244, 0.155)
Cd	0.109 (0.019, 0.237)*	0.089 (−0.043, 0.221)	0.117 (0.028, 0.263)*	0.061 (−0.045, 0.167)	0.066 (−0.045, 0.176)	0.109 (0.009, 0.226)*	0.026 (−0.104, 0.157)	0.002 (−0.132, 0.135)	0.026 (−0.119, 0.171)
Hg	0.157 (0.020, 0.293)*	0.177 (0.040, 0.313)*	0.157 (0.002, 0.313)*	0.033 (−0.081, 0.148)	0.035 (−0.082, 0.152)	0.013 (−0.117, 0.142)	0.018 (−0.123, 0.159)	0.023 (−0.118, 0.164)	0.112 (−0.044, 0.268)
As	0.014 (−0.134, 0.163)	0.006 (−0.145, 0.158)	−0.049 (−0.217, 0.120)	0.016 (−0.106, 0.138)	0.010 (−0.116, 0.137)	−0.055 (−0.191, 0.082)	0.116 (−0.033, 0.264)	0.119 (−0.032, 0.269)	0.182 (−0.019, 0.346)

Immune cells: lymphocyte, neutrophil and monocyte; heavy metals: Pb, Cd, Hg and As. Data of heavy metals and immune cell counts were ln-transformed to normalize the distribution. Data in this table are presented as estimates (95% confidence intervals). The estimates (β value) were calculated by linear regression models. Model 1 represents a crude model without any adjustment; model 2 was adjusted for child age, gender and BMI; model 3 was adjusted for child age, gender and BMI, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m (m) of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop. Values of $*p < 0.05$ was considered statistically significant.

Table 3

Associations between heavy metals and pro-inflammatory cytokines (n = 147).

	IL-1 β			IL-6			IL-8			TNF- α		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Pb	0.225 (0.002, 0.447)*	0.187 (−0.038, 0.411)	0.186 (−0.083, 0.454)	0.287 (0.056, 0.518)*	0.264 (0.038, 0.491)*	0.327 (0.036, 0.617)*	−0.110 (−0.377, 0.158)	−0.134 (−0.398, 0.130)	0.048 (−0.277, 0.373)	−0.041 (−0.247, 0.166)	−0.082 (−0.289, 0.125)	−0.179 (−0.440, 0.082)
Cd	0.172 (0.013, 0.357)*	0.165 (0.023, 0.352)*	0.151 (−0.044, 0.346)	0.071 (−0.124, 0.267)	0.059 (−0.134, 0.253)	0.055 (−0.161, 0.271)	−0.045 (−0.268, 0.177)	−0.131 (−0.352, 0.089)	−0.053 (−0.289, 0.183)	−0.112 (−0.282, 0.058)	−0.126 (−0.299, 0.046)	−0.149 (−0.339, 0.040)
Hg	−0.099 (−0.300, 0.102)	−0.094 (−0.294, 0.105)	−0.132 (−0.344, 0.080)	−0.198 (−0.406, −0.010)	−0.179 (−0.381, 0.023)	−0.245 (−0.639, 0.082)	−0.158 (−0.396, 0.080)	−0.110 (−0.343, 0.124)	−0.094 (−0.350, 0.162)	−0.062 (−0.246, 0.122)	−0.062 (−0.245, 0.121)	−0.143 (−0.349, 0.063)
As	0.008 (−0.207, 0.223)	−0.037 (−0.253, 0.179)	−0.056 (−0.282, 0.170)	0.026 (−0.199, 0.251)	−0.056 (−0.276, 0.164)	−0.114 (−0.362, 0.133)	−0.059 (−0.314, 0.196)	−0.094 (−0.346, 0.157)	−0.111 (−0.382, 0.160)	0.008 (−0.188, 0.204)	−0.030 (−0.228, 0.167)	−0.139 (−0.358, 0.079)

Pro-inflammatory cytokines: IL-1 β , IL-6, IL-8 and TNF- α ; IL: interleukin; TNF: tumor necrosis factor. Heavy metals: Pb, Cd, Hg and As. Data of heavy metals and pro-inflammatory cytokines were ln-transformed to normalize the distribution. Data in this table are presented as estimates (95% confidence intervals). The estimates (β value) were calculated by linear regression models. Model 1 represents a crude model without any adjustment; model 2 was adjusted for child age, gender and BMI; model 3 was adjusted for child age, gender and BMI, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m (m) of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop. Values of $*p < 0.05$ was considered statistically significant.

Table 4

Associations between heavy metals and anti-inflammatory cytokines (n = 147).

	IL-1RA			IL-4			IL-10			IL-13		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Pb	−0.336 (−0.583, −0.089)**	−0.392 (−0.639, −0.144)**	−0.188 (−0.499, 0.124)	−0.056 (−0.178, 0.067)	−0.096 (−0.216, 0.025)	−0.118 (−0.270, 0.033)	−0.005 (−0.290, 0.279)	−0.005 (−0.296, 0.286)	0.075 (−0.286, 0.437)	−0.090 (−0.253, 0.073)	−0.123 (−0.288, 0.042)	−0.086 (−0.289, 0.117)
Cd	−0.181 (−0.389, −0.027)*	−0.207 (−0.419, −0.006)*	−0.150 (−0.377, 0.076)	−0.026 (−0.127, 0.076)	−0.043 (−0.144, 0.058)	−0.072 (−0.182, 0.039)	−0.167 (−0.401, 0.067)	−0.139 (−0.381, 0.104)	−0.182 (−0.443, 0.079)	−0.007 (−0.143, 0.128)	−0.020 (−0.160, 0.119)	−0.033 (−0.181, 0.115)
Hg	−0.286 (−0.507, −0.065)*	−0.288 (−0.505, −0.070)*	−0.163 (−0.426, 0.100)	−0.082 (−0.191, 0.027)	−0.082 (−0.189, 0.024)	−0.106 (−0.255, 0.014)	−0.102 (−0.355, 0.151)	−0.125 (−0.381, 0.131)	−0.029 (−0.314, 0.256)	−0.114 (−0.258, 0.031)	−0.116 (−0.262, 0.030)	−0.101 (−0.261, 0.058)
As	−0.173 (−0.412, −0.067)*	−0.223 (−0.464, −0.019)*	−0.138 (−0.399, 0.123)	−0.023 (−0.140, 0.094)	−0.043 (−0.159, 0.072)	−0.070 (−0.198, 0.058)	−0.177 (−0.446, 0.091)	−0.190 (−0.465, 0.085)	−0.162 (−0.463, 0.139)	0.023 (−0.133, 0.178)	0.012 (−0.146, 0.171)	−0.008 (−0.179, 0.162)

Anti-inflammatory cytokines: IL-1RA, IL-4, IL-10 and IL-13; RA: receptor antagonist. Heavy metals: Pb, Cd, Hg and As. Data of heavy metals and pro-inflammatory cytokines were ln-transformed to normalize the distribution. Data in this table are presented as estimates (95% confidence intervals). The estimates (β value) were calculated by linear regression models. Model 1 represents a crude model without any adjustment; model 2 was adjusted for child age, gender and BMI; model 3 was adjusted for child age, gender and BMI, distance of the road from the residence, milk consumption frequency, parents' education level, daily smoking by family members, parents' residence time, frequency of washing hands before meals, child born locally (Guiyu or Haojiang), child e-waste contact, ventilation of house, e-waste pollution within 50 m (m) of residence, maternal occupation associated with e-waste dismantling, residence used as a workshop. Values of $*p < 0.05$, $**p < 0.01$ were considered statistically significant.

neutrophils in the bone marrow and spleens, while the significantly higher neutrophil count was observed in the peripheral blood of Cd-exposed rats (El-Boshy et al., 2017; Zhang et al., 2016b). Our

finding is also in line with a prior animal study showing enhanced neutrophil counts concerning increasing Hg exposure (Schaefer et al., 2011), but not identical with a recent cohort study

reporting no significant correlation of neutrophil counts with the Hg concentration (Oulhote et al., 2017).

Due to the difficulty of evaluating tissue-resident macrophages, as well as the rarity of dendritic cells in human blood (Ohradanova-Repic et al., 2016), we could only analyze monocyte counts in child peripheral blood. However, a significant alteration in monocyte amount was not found in the present study. Despite that, our finding of reduced lymphocyte count in exposed populations is in accordance with several studies (Boscolo et al., 2000; Mishra et al., 2010; Oulhote et al., 2017). It is well known that the migration of circulating lymphocytes plays a more prominent role in eliminating pathogenic microorganisms or noxious stimuli in chronic inflammation; hence, the decreased lymphocyte quantity is not conducive to the immune system in response to harmful conditions (Fortier et al., 2008; Sakai and Kobayashi, 2015; Steffensen et al., 1994; Valentino et al., 2007). A decrease in lymphocyte count might hint that the proliferation of lymphocytes is disturbed or suppressed, which could lead to an ineffective immune function of children in e-waste areas.

Heavy metals upset the balance of the pro- and anti-inflammatory processes. Our study demonstrated that children from the e-waste recycling area have elevated pro-inflammatory IL-1 β and IL-6, but lower anti-inflammatory IL-1RA and IL-13 levels. No difference in IL-8, TNF- α , IL-4, and IL-10 production was found between two groups, which is not surprising because the release of cytokines may partially be attributed to stimulants, but could be driven by genetics, cytokine half-lives or other unexpected confounders. The half-life of each biomarker is shown in Table S4 in Supplementary Materials. IL-1 β is mainly secreted by monocytes, tissue macrophages and dendritic cells. It exerts diverse effects on multiple cell types, culminating in broad inflammation, although often being at best only slightly elevated in human inflammatory diseases even under conditions of severe pathology (Dinarello, 2009; Lachmann et al., 2009). IL-1RA is expressed by various cells in an inflammatory environment and is regarded as an essential negative feedback mechanism to limit inflammation mediated by IL-1 β (Roerink et al., 2017). IL-13 is primarily generated by T helper type 2 (Th2) cells and excessively produced in allergy, asthma and other chronic inflammatory diseases (Alasandagutti et al., 2017; Fujii et al., 2017). At present, we cannot identify far-reaching health consequences, such as chronic diseases and malignancies in children from e-waste areas. According to the imbalance of inflammatory cytokines, our finding suggests that children living in the e-waste areas gravitate towards an inflammatory status or at least possess a relatively high inflammation risk.

We noticed that abnormal cytokine expression, including of IL-1 β , IL-6 and IL-1RA, is dependent on heavy metal exposure. Our observation indicates that Pb exposure induces IL-1 β and IL-6 but suppresses IL-1RA production. The association between Pb and IL-1 β can be weakened when adjusting individuals' characteristics and social-behavioral factors, indicating that these factors may contribute to IL-1 β production. In parallel with our results, previous research in both humans and animals has also shown IL-6 is over-expressed following Pb exposure (Kayaalti et al., 2011; Mishra et al., 2003; Struzynska et al., 2007; Wang et al., 2016). IL-6 is proposed as a predictor for malignancy since elevated IL-6 level is closely associated with tumorigenic progression; in addition, dysregulated IL-6 synthesis plays a pathological role in chronic inflammation and autoimmunity (Landskron et al., 2014; Tanaka et al., 2014; Taniguchi and Karin, 2014). Moreover, Pb, Cd, Hg and As exposure all exhibit inhibitory contribution to IL-1RA synthesis. With regard to the associations between all 4 metals and IL-1RA, they did not exist in model 3, suggesting that they may be hampered by social-behavioral factors. There unfortunately is no supporting evidence for the correlation of their exposure and IL-1RA secretion. In this

case, our finding is novel and could be an opportunity for future research. As a consequence of the alterations in pro-inflammatory IL-1 β , IL-6 and anti-inflammatory IL-1RA, we speculated that individuals exposed to Pb, Cd, Hg and As might sustain a potential threat by immune imbalance and inflammatory conditions.

Exposure to heavy metals could be harmful for Guiyu children. In general, immune function may be enhanced, as a protective mechanism, to respond to danger signals derived from endogenous and exogenous molecules after acute exposure to occupational and environmental pollutants (Franklin et al., 2015; Steinvil et al., 2008). However, long-term exposure to heavy metals (either low-dose or high-dose) uniformly causes immunosuppression and directly interferes with the sensitization of the immune system to antigens (Habtezion, 2015; Hoffmann et al., 2009; Sorvari et al., 2007; Viehmann et al., 2015). So to some extent, our observation is in agreement with previous findings that exposure to heavy metals prompts several subtle changes in perturbing immune regulation. Continuous heavy metal exposures could inevitably impair immune function, subsequently resulting in the occurrence of the disease. This however needs further health surveillance and assessment.

Several limitations of our study should be stated. Considering the sample size, we explored the impact of heavy metal exposure on cells and cytokines involved in the immune regulatory and inflammatory processes after merging all data from both groups. Therefore, a few unknown confounders will interfere with our established linear models. Many other toxic compounds and pollutants, for example, persistent organic pollutants are also a major concern of the e-waste dismantling area (Leung et al., 2008; Xu et al., 2013). It is challenging to run all items of tests in this study due to the insufficient volume of blood samples. Some customs and individual habits may affect immune homeostasis and inflammatory activation in children. However, we failed to provide the evidence for the analysis in this regard. Moreover, since inflammation is generally accompanied by tissue injury, monitoring tissue-resident macrophages or mast cells would be very important in the assessment of different inflammatory period. Thus, additional studies are sorely needed to better understand the influence of heavy metal exposure on the occurrence and development of inflammation and other diseases in future investigations.

5. Conclusions

This study identified the alterations of immune cells and cytokines in preschool children exposed to heavy metals Pb, Cd, Hg and As. Children living in the e-waste recycling area had higher levels of heavy metals. Decreased blood lymphocyte amount was observed in children from the e-waste recycling area, which might suggest their immune function is either disturbed or suppressed. Elevated pro-inflammatory IL-1 β and IL-6, and declined anti-inflammatory IL-1RA and IL-13 indicate that Guiyu children had dysregulated immune response and high inflammation risk. Association analysis reveals that exposure to Pb, Cd, Hg and As is likely to contribute to the alterations in neutrophils, IL-1 β , IL-6 and IL-1RA, prompting adverse effects on immune response and inflammatory regulation. Our finding of the inverse association between IL-1RA and heavy metals is novel and could be an opportunity for future research.

Contributions of each author

Xijin Xu designed the study, searched the literature, supervised data acquisition, revised the manuscript, and funded the study. Yu Zhang contributed to the work for sample and questionnaire collections, experiment implementation, and data analysis, and wrote the manuscript. Xia Huo assisted in the study design, data

interpretation, and edited the manuscript. Xueling Lu and Zhijun Zeng helped in the process of experiment for techniques. Marijke M. Faas provided constructive comments and revised the manuscript. All authors approved the final version for submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.127257>.

References

- Afridi, H.I., Kazi, T.G., Kazi, N., et al., 2013. Evaluation of status of arsenic, cadmium, lead and zinc levels in biological samples of normal and arthritic patients of age groups (46 - 60) and (61 - 75) years. *Clin. Lab.* 59, 143–153.
- Al Bakheet, S.A., Attafi, I.M., Maayah, Z.H., et al., 2013. Effect of long-term human exposure to environmental heavy metals on the expression of detoxification and DNA repair genes. *Environ. Pollut.* 181, 226–232.
- Alasandagutti, M.L., Ansari, M.S., Sagurthi, S.R., et al., 2017. Role of IL-13 genetic variants in signalling of asthma. *Inflammation* 40, 566–577.
- Arbuckle, T.E., Liang, C.L., Morisset, A.S., et al., 2016. Maternal and fetal exposure to cadmium, lead, manganese and mercury: the MIREC study. *Chemosphere* 163, 270–282.
- Arora, M., Weuve, J., Schwartz, J., et al., 2009. Association of environmental cadmium exposure with periodontal disease in U.S. adults. *Environ. Health Perspect.* 117, 739–744.
- Bae, J.H., Shim, J.H., Cho, Y.S., 2014. Chemical regulation of signaling pathways to programmed necrosis. *Arch. Pharm. Res. (Seoul)* 37, 689–697.
- Boscolo, P., Di Gioacchino, M., Sabbioni, E., et al., 2000. Lymphocyte subpopulations, cytokines and trace elements in asymptomatic atopic women exposed to an urban environment. *Life Sci.* 67, 1119–1126.
- Brucker, N., Moro, A., Charao, M., et al., 2015. Relationship between blood metals and inflammation in taxi drivers. *Clin. Chim. Acta* 444, 176–181.
- Cao, J., Xu, X., Zhang, Y., et al., 2018. Increased memory T cell populations in Pb-exposed children from an e-waste-recycling area. *Sci. Total Environ.* 616–617, 988–995.
- Cavaleri, F., 2015. Review of Amyotrophic Lateral Sclerosis, Parkinson's and Alzheimer's diseases helps further define pathology of the novel paradigm for Alzheimer's with heavy metals as primary disease cause. *Med. Hypotheses* 85, 779–790.
- Chen, A., Dietrich, K.N., Huo, X., et al., 2011. Developmental neurotoxicants in e-waste: an emerging health concern. *Environ. Health Perspect.* 119, 431–438.
- Cho, Y., Song, M.K., Kim, T.S., et al., 2017. Identification of novel cytokine biomarkers of hexanal exposure associated with pulmonary toxicity. *Environ. Pollut.* 229, 810–817.
- Dai, Y., Huo, X., Zhang, Y., et al., 2017. Elevated lead levels and changes in blood morphology and erythrocyte CR1 in preschool children from an e-waste area. *Sci. Total Environ.* 592, 51–59.
- Deng, X., Wang, L., Elm, M.S., et al., 2005. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. *Am. J. Pathol.* 166, 93–106.
- Di Lorenzo, L., Silvestroni, A., Martino, M.G., et al., 2006. Evaluation of peripheral blood neutrophil leucocytes in lead-exposed workers. *Int. Arch. Occup. Environ. Health* 79, 491–498.
- Dinarello, C.A., 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.
- El-Boshy, M., Ashshi, A., Gaith, M., et al., 2017. Studies on the protective effect of the artichoke (*Cynara scolymus*) leaf extract against cadmium toxicity-induced oxidative stress, hepatorenal damage, and immunosuppressive and hematological disorders in rats. *Environ. Sci. Pollut. Res. Int.* 24, 12372–12383.
- Ferrero-Miliani, L., Nielsen, O.H., Andersen, P.S., et al., 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 β generation. *Clin. Exp. Immunol.* 147, 227–235.
- Fortier, M., Omara, F., Bernier, J., et al., 2008. Effects of physiological concentrations of heavy metals both individually and in mixtures on the viability and function of peripheral blood human leukocytes in vitro. *J. Toxicol. Environ. Health Part A* 71, 1327–1337.
- Fournier, B.M., Parkos, C.A., 2012. The role of neutrophils during intestinal inflammation. *Mucosal Immunol.* 5, 354–366.
- Franklin, B.A., Brook, R., Arden Pope 3rd, C., 2015. Air pollution and cardiovascular disease. *Curr. Probl. Cardiol.* 40, 207–238.
- Fujii, U., Miyahara, N., Taniguchi, A., et al., 2017. Effect of a retinoid X receptor partial agonist on airway inflammation and hyperresponsiveness in a murine model of asthma. *Respir. Res.* 18, 23.
- Giovanni, M., Yue, J., Zhang, L., et al., 2015. Pro-inflammatory responses of RAW264.7 macrophages when treated with ultralow concentrations of silver, titanium dioxide, and zinc oxide nanoparticles. *J. Hazard Mater.* 297, 146–152.
- Goecke, H., Forssmann, U., Uguccioni, M., et al., 2000. Macrophages infiltrating the tissue in chronic pancreatitis express the chemokine receptor CCR5. *Surgery* 128, 806–814.
- Grant, K., Goldizen, F.C., Sly, P.D., et al., 2013. Health consequences of exposure to e-waste: a systematic review. *Lancet Glob Health* 1, e350–e361.
- Guo, Y., Huo, X., Li, Y., et al., 2010. Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China. *Sci. Total Environ.* 408, 3113–3117.
- Habtezion, A., 2015. Inflammation in acute and chronic pancreatitis. *Curr. Opin. Gastroenterol.* 31, 395–399.
- Hamilton, J.A., 2008. Colony-stimulating factors in inflammation and autoimmunity. *Nat. Rev. Immunol.* 8, 533–544.
- Hausser, G., Ludewig, B., Gelderblom, H.R., et al., 1997. Monocyte-derived dendritic cells represent a transient stage of differentiation in the myeloid lineage. *Immunobiology* 197, 534–542.
- Hoffmann, B., Moebus, S., Dragano, N., et al., 2009. Chronic residential exposure to particulate matter air pollution and systemic inflammatory markers. *Environ. Health Perspect.* 117, 1302–1308.
- Huo, X., Peng, L., Xu, X., et al., 2007. Elevated blood lead levels of children in Guiyu, an electronic waste recycling town in China. *Environ. Health Perspect.* 115, 1113–1117.
- Ingersoll, M.A., Platt, A.M., Potteaux, S., et al., 2011. Monocyte trafficking in acute and chronic inflammation. *Trends Immunol.* 32, 470–477.
- Iyer, S.S., Pulsikens, W.P., Sadler, J.J., et al., 2009. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20388–20393.
- Jin, Y., Liu, L., Zhang, S., et al., 2016. Cadmium exposure to murine macrophages decreases their inflammatory responses and increases their oxidative stress. *Chemosphere* 144, 168–175.
- Kayaaltı, Z., Tekin, D., Aliyev, V., et al., 2011. Effects of the interleukin-6 (IL-6) polymorphism on toxic metal and trace element levels in placental tissues. *Sci. Total Environ.* 409 (23), 4929–4933.
- Kolaczowska, E., Kubes, P., 2013. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 13, 159–175.
- Kumar, S., Jack, R., 2006. Origin of monocytes and their differentiation to macrophages and dendritic cells. *J. Endotoxin Res.* 12, 278–284.
- Lachmann, H.J., Lowe, P., Felix, S.D., et al., 2009. In vivo regulation of interleukin 1 β in patients with cryopyrin-associated periodic syndromes. *J. Exp. Med.* 206, 1029–1035.
- Lana, R.M., Riback, T.L.S., Lima, T.F.M., et al., 2017. Socioeconomic and demographic characterization of an endemic malaria region in Brazil by multiple correspondence analysis [published correction appears in *Malar. J.* 16, 397, 2017 Oct 11;16(1):408].
- Landskron, G., De la Fuente, M., Thuwajit, P., et al., 2014. Chronic inflammation and cytokines in the tumor microenvironment. *J. Immunol. Res.* 2014, 149185.
- Lau, W.K., Liang, P., Man, Y.B., et al., 2014. Human health risk assessment based on trace metals in suspended air particulates, surface dust, and floor dust from e-waste recycling workshops in Hong Kong, China. *Environ. Sci. Pollut. Res. Int.* 21, 3813–3825.
- Leung, A.O.W., Duzgorenaydin, N.S., Cheung, K.C., et al., 2008. Heavy metals concentrations of surface dust from e-waste recycling and its human health implications in southeast China. *Environ. Sci. Technol.* 42, 2674–2680.
- Li, R., Kou, X., Geng, H., et al., 2015. Mitochondrial damage: an important mechanism of ambient PM_{2.5} exposure-induced acute heart injury in rats. *J. Hazard Mater.* 287, 392–401.
- Liu, R., Xu, X., Zhang, Y., et al., 2016. Thyroid hormone status in umbilical cord serum is positively associated with male anogenital distance. *J. Clin. Endocrinol. Metab.* 101, 3378–3385.
- Lu, S.Y., Zhang, H.M., Sojinu, S.O., et al., 2015. Trace elements contamination and human health risk assessment in drinking water from Shenzhen, China. *Environ. Monit. Assess.* 187, 4220.
- Mishra, K.P., Singh, V.K., Rani, R., et al., 2003. Effect of lead exposure on the immune response of some occupationally exposed individuals. *Toxicology* 188, 251–259.
- Mishra, K.P., Rani, R., Yadav, V.S., et al., 2010. Effect of lead exposure on lymphocyte subsets and activation markers. *Immunopharmacol. Immunotoxicol.* 32, 446–449.
- Monnet-Tschudi, F., Zurich, M.G., Boschat, C., et al., 2006. Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev.*

- Environ. Health 21, 105–117.
- Morcillo, P., Esteban, M.Á., Cuesta, A., 2016. Heavy metals produce toxicity, oxidative stress and apoptosis in the marine teleost fish SAF-1 cell line. *Chemosphere* 144, 225–233.
- Ohradanova-Repic, A., Machacek, C., Fischer, M.B., et al., 2016. Differentiation of human monocytes and derived subsets of macrophages and dendritic cells by the HLDA10 monoclonal antibody panel. *Clin. Trans. Immunol.* 5, e55.
- Olszowski, T., Baranowska-Bosiacka, I., Gutowska, I., et al., 2012. Pro-inflammatory properties of cadmium. *Acta Biochim. Pol.* 59, 475–482.
- Oulhote, Y., Shamim, Z., Kielsen, K., et al., 2017. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. *Reprod. Toxicol.* 68, 207–214.
- Roerink, M.E., van der Schaaf, M.E., Dinarello, C.A., et al., 2017. Interleukin-1 as a mediator of fatigue in disease: a narrative review. *J. Neuroinflammation* 14, 16.
- Rowley, B., Monestier, M., 2005. Mechanisms of heavy metal-induced autoimmunity. *Mol. Immunol.* 42, 833–838.
- Rzymiski, P., Tomczyk, K., Rzymiski, P., et al., 2015. Impact of heavy metals on the female reproductive system. *Ann. Agric. Environ. Med.* 22, 259–264.
- Sakai, Y., Kobayashi, M., 2015. Lymphocyte 'homing' and chronic inflammation. *Pathol. Int.* 65, 344–354.
- Schaefer, A.M., Stavros, H.C., Bossart, G.D., et al., 2011. Associations between mercury and hepatic, renal, endocrine, and hematological parameters in Atlantic bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of Florida and South Carolina. *Arch. Environ. Contam. Toxicol.* 61, 688–695.
- Schenkein, H.A., Loos, B.G., 2013. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. *J. Clin. Periodontol.* 40, 51–69.
- Silver, M.K., Arain, A.L., Shao, J., et al., 2018. Distribution and predictors of 20 toxic and essential metals in the umbilical cord blood of Chinese newborns. *Chemosphere* 210, 1167–1175.
- Sorvari, J., Rantala, L.M., Rantala, M.J., et al., 2007. Heavy metal pollution disturbs immune response in wild ant populations. *Environ. Pollut.* 145, 324–328.
- Steffensen, I.L., Mesna, O.J., Andruchow, E., et al., 1994. Cytotoxicity and accumulation of Hg, Ag, Cd, Cu, Pb and Zn in human peripheral T and B lymphocytes and monocytes in vitro. *Gen. Pharmacol.* 25, 1621–1633.
- Steinvil, A., Kordova-Biezuner, L., Shapira, I., et al., 2008. Short-term exposure to air pollution and inflammation-sensitive biomarkers. *Environ. Res.* 106, 51–61.
- Struzynska, L., Dabrowska-Bouta, B., Koza, K., et al., 2007. Inflammation-like glial response in lead-exposed immature rat brain. *Toxicol. Sci.* 95, 156–162.
- Tanaka, T., Narazaki, M., Kishimoto, T., 2014. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* 6, a016295.
- Taniguchi, K., Karin, M., 2014. IL-6 and related cytokines as the critical lymphins between inflammation and cancer. *Semin. Immunol.* 26, 54–74.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., et al., 2012. Heavy metal toxicity and the environment. *EXS* 101, 133–164.
- Tseng, J.C., Kung, A.L., 2012. In vivo imaging of inflammatory phagocytes. *Chem. Biol.* 19, 1199–1209.
- Tseng, J.C., Kung, A.L., 2013. In vivo imaging method to distinguish acute and chronic inflammation. *J. Vis. Exp.* 16, 78.
- Valentino, M., Rapisarda, V., Santarelli, L., et al., 2007. Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers. *Hum. Exp. Toxicol.* 26, 551–556.
- Viehmann, A., Hertel, S., Fuks, K., et al., 2015. Long-term residential exposure to urban air pollution, and repeated measures of systemic blood markers of inflammation and coagulation. *Occup. Environ. Med.* 72, 656–663.
- Wang, S., Shi, X., 2001. Molecular mechanisms of metal toxicity and carcinogenesis. *Mol. Cell. Biochem.* 222, 3–9.
- Wang, H., Li, D., Hu, Z., et al., 2016. Protective effects of green tea polyphenol against renal injury through ROS-mediated JNK-MAPK pathway in lead exposed rats. *Mol. Cell* 39, 508–513.
- Wessling-Resnick, M., 2010. Iron homeostasis and the inflammatory response. *Annu. Rev. Nutr.* 30, 105–122.
- Wu, Y., Antony, S., Meitzler, J.L., et al., 2014. Molecular mechanisms underlying chronic inflammation-associated cancers. *Canc. Lett.* 345, 164–173.
- Wu, Y., Zhang, H., Liu, G., et al., 2016. Concentrations and health risk assessment of trace elements in animal-derived food in southern China. *Chemosphere* 144, 564–570.
- Xu, P., Tao, B., Li, N., et al., 2013. Levels, profiles and source identification of PCDD/Fs in farmland soils of Guiyu, China. *Chemosphere* 91, 824–831.
- Xu, X., Chen, X., Zhang, J., et al., 2015. Decreased blood hepatitis B surface antibody levels linked to e-waste lead exposure in preschool children. *J. Hazard Mater.* 298, 122–128.
- Xu, L., Huo, X., Liu, Y., et al., 2020. Hearing loss risk and DNA methylation signatures in preschool children following lead and cadmium exposure from an electronic waste recycling area. *Chemosphere* 246, 125829.
- Zeng, X., Xu, X., Zheng, X., et al., 2017. Heavy metals in PM 2.5 and in blood, and children's respiratory symptoms and asthma from an e-waste recycling area. *Environ. Pollut.* 210, 346–353.
- Zeng, X., Xu, X., Qin, Q., et al., 2019. Heavy metal exposure has adverse effects on the growth and development of preschool children. *Environ. Geochem. Health* 41, 309–321.
- Zhang, Y., Huo, X., Cao, J., et al., 2016a. Elevated lead levels and adverse effects on natural killer cells in children from an electronic waste recycling area. *Environ. Pollut.* 213, 143–150.
- Zhang, Y., Yu, X., Sun, S., et al., 2016b. Cadmium modulates hematopoietic stem and progenitor cells and skews toward myelopoiesis in mice. *Toxicol. Appl. Pharmacol.* 313, 24–34.
- Zheng, L., Wu, K., Li, Y., et al., 2008. Blood lead and cadmium levels and relevant factors among children from an e-waste recycling town in China. *Environ. Res.* 108, 15–22.
- Zheng, X., Huo, X., Zhang, Y., et al., 2019. Cardiovascular endothelial inflammation by chronic coexposure to lead (Pb) and polycyclic aromatic hydrocarbons from preschool children in an e-waste recycling area. *Environ. Pollut.* 246, 587–596.