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Increased memory T cell populations in Pb-exposed children from an e-waste-recycling area

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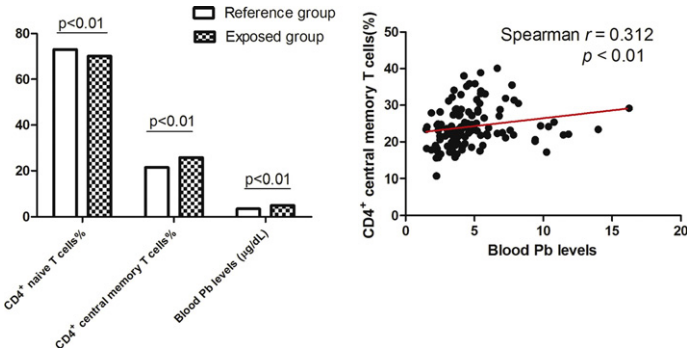
HIGHLIGHTS

- Percentages of $CD4^+$ central memory T cells are increased in e-waste-exposed children.
- Blood Pb levels are higher in e-waste-exposed children.
- Blood Pb levels are positively associated with percentages of $CD4^+$ central memory T cells.
- Pb exposure may facilitate development of $CD4^+$ central T cell memory in children.

GRAPHICAL ABSTRACT



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ABSTRACT

Chronic exposure to heavy metals could affect cell-mediated immunity. The aim of this study was to explore the status of memory T cell development in preschool children from an e-waste recycling area. Blood lead (Pb) levels, peripheral T cell subpopulations, and serum levels of cytokines (IL-2/IL-7/IL-15), relevant to generation and homeostasis of memory T cells were evaluated in preschool children from Guiyu (e-waste-exposed group) and Haojiang (reference group). The correlations between blood Pb levels and percentages of memory T cell subpopulations were also evaluated. Guiyu children had higher blood Pb levels and increased percentages of $CD4^+$ central memory T cells and $CD8^+$ central memory T cells than in the Haojiang group. Moreover, blood Pb levels were positively associated with the percentages of $CD4^+$ central memory T cells. In contrast, Pb exposure contributed marginally in the change of percentages of $CD8^+$ central memory T cells in children. There was no significant difference in the serum cytokine levels between the e-waste-exposed and reference children. Taken together, preschool children from an e-waste recycling area suffer from relatively higher levels of Pb exposure, which might facilitate the development of $CD4^+$ central memory T cells in these children.

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1. Introduction

The amount of e-waste has rapidly increased worldwide in recent years due to rapid up-grading of electronic products. A sizable amount of e-waste is transported to developing countries or emerging industrialized countries for the recycling of valuable metals, such as copper and gold. Crude recycling operations result in extensive release of toxicants, derived from e-waste, into the local environment. For example, Guiyu, the largest e-waste recycling town in China, has witnessed serious heavy metal contamination in air, water, soil, plants and sediment (Alabi et al., 2012; Leung et al., 2008; Wang and Guo, 2006). Higher levels of heavy metals, such as Pb, cadmium, and chromium, have also been detected in the blood of local neonatal and preschool children (Xu et al., 2017) (Dai et al., 2017; Guo et al., 2010; Li et al., 2008; Lin et al., 2016; Xu et al., 2016; Zhang et al., 2011).

A series of studies suggests that heavy metals can regulate T lymphocyte functions. Non-toxic levels of sodium arsenite (As, 0.25–2 μM) can block human T cell cycling and prevent T cell proliferation and IL-2 expression following in vitro CD3/CD28 activation (Morzadec et al., 2012), whereas higher levels of As not only suppress $CD4^+$ and $CD8^+$ T cell proliferation but also reduce the expression of CD25 and CD69 (activation markers) in both human T cell subtypes (Tenorio and Saavedra, 2005). Further, As can promote apoptosis of human and murine T lymphocytes by activation of multiple death-induction pathways (Gupta et al., 2003; Hossain et al., 2000). In addition to As, cadmium can also promote T cell apoptosis by induction of oxidative stress, and reduce T cell-specific cytokine release (Grazia Cifone et al., 1989; Pathak and Khandelwal, 2008). Mercury, however, can stimulate T cell immune responses and promote autoimmune inflammation (Schiraldi and Monestier, 2009; Shenker et al., 1998; Shenker et al., 2002; Vas and Monestier, 2008). Pb, another common environmental pollutant, can also widely regulate T cell survival and function. For example, low levels of Pb directly target CD11c-enriched antigen-presenting cells to promote antigen-specific $CD4^+$ T cell proliferation in vitro (Farrer et al., 2005). At concentrations that do not alter T cell proliferation, Pb can differentially regulate cytokine production from resting and antigen-activated $CD4^+$ T cells (Shen et al., 2001). Pb also disrupts protein biosynthesis of interferon gamma (IFN-γ) in Th1 cells, leading to greater Th2 cytokine production (Heo et al., 2007). Together, heavy metals, at different concentrations, may exert multiple regulatory roles on T cell activation, proliferation and effector function.

In a primary immune response, naïve T cells are activated and differentiate into effector T cells. At the end stage of a primary immune response, a small fraction of effector T cells will differentiate into memory T cells (MacLeod et al., 2010). Although the specific mechanisms of fate choice of central memory T cells vs effector memory T cells are still under investigation (Chandok and Farber, 2004; Sallusto et al., 2004; Tuuminen et al., 2007), strong T cell receptor signaling initiated by antigen binding (Williams et al., 2008) and sufficient T cell receptor-antigen interaction are essential for memory T cell development (Kim et al., 2013). Moreover, the cytokines such as IL-2, IL-7, and IL-15 also play an important role in memory T cell differentiation and survival (Jaleco et al., 2003; Li et al., 2003; Prlic et al., 2002; Seddon et al., 2003). Because heavy metals such as Pb can affect T cell activation, proliferation and cytokine secretion, it might also affect memory T cell generation and homeostasis. Interestingly, a previous study reported a decrease of $CD3^+CD45RO^+$ memory T cells in adult workers who were occupationally exposed to high levels of Pb (Sata et al., 1998). However, data that evaluate relationship between blood Pb levels and development of memory T cells is scarce, especially in preschool children. Because preschool children have a lower tolerance than adults to environmental toxicants (Dietert, 2008), and experience rapid maturation of memory immunity, Pb exposure might disturb the differentiation of memory T cells in young children. Our previous studies showed that Pb exposure is associated with lower titers of several types of antibodies in children from an e-waste recycling area (Lin et al., 2017; Lin et al., 2016; Xu et al., 2015), suggesting that Pb exposure might affect generation or maintenance of humoral immunity memory in these children. Considering the role of memory T cells in prevention of infectious diseases in young children, it is crucial to know the status of memory T cell immunity in children exposed to Pb. In this study, percentages of peripheral memory T cell subpopulations were investigated in preschool children from Guiyu, a well-known e-waste recycling area and Haojiang, a reference area, in China. The relationship between blood Pb levels and percentages of peripheral memory T cells was also explored in preschool children.

2. Materials and methods

2.1. Study population

A cross-sectional study was performed in Guiyu, a large e-waste crude recycling area, and Haojiang, a reference area in China. Haojiang was selected as the reference area because there is no e-waste contamination, and the population displays a similar culture, diet, and ethnicity as in Guiyu (Xu et al., 2015). A total of 118 local kindergarten children (62 from Guiyu and 56 from Haojiang), from 3 to 7 years old, were recruited in 2014 for flow cytometry analysis, routine blood tests and evaluation of serum cytokine levels (Table 1). The subjects included in this study met the following criteria: 1) no consumption of drugs or antibiotics within 1 month before sample collection 2) no absentee record for flu or other infectious diseases within 1 month before sample collection 3) physically healthy based on the physician examination when samples were collected and 4) at least one year of residence in the local areas. Information about each child's age, sex, life style, medical history, pollutant exposure, and parents' socioeconomic status were also collected by a detailed questionnaire. All parents and guardians were fully informed of the study and signed the written consent form. The study proposal was screened and approved by the Medical Ethnic Committee of Shantou University Medical College.

Table 1
Baseline characteristics of children in the study.

Variables	Reference ($n = 56$)	Exposed ($n = 62$)	p
Age (years, mean ± SD)	4.58 ± 0.87	5.05 ± 0.55	0.001
Gender, boys/girls, count	33/23	29/33	0.187
Height (cm, mean ± SD)	107.02 ± 7.29	109.29 ± 5.93	0.064
Weight (kg, mean ± SD)	17.80 ± 2.67	17.30 ± 3.25	0.204
Blood Pb levels (μg/dL) (GM ± S_{logx})	3.60 ± 0.21	5.06 ± 0.17	0.000
≤5 μg/dL, counts	49/56	32/62	0.000
>5 μg/dL, counts	7/56	30/62	0.000
Leukocyte counts ($\times 10^9$/L) (mean ± SD)	9.02 ± 2.21	8.68 ± 1.70	0.350
Lymphocyte counts ($\times 10^9$/L) (mean ± SD)	4.12 ± 1.10	3.73 ± 0.98	0.041
Lymphocytes in leukocyte pool (%) (mean ± SD)	46.88 ± 10.45	43.21 ± 8.13	0.035
Average monthly income (RMB) [n(%)]			0.002
<1000	0 (0)	2 (3.23)	
1000–3000	4 (7.14)	11 (17.74)	
3001–5000	35 (62.5)	21 (33.87)	
>5000	31 (55.36)	14 (22.58)	
Daily cigarette consumption in family [n(%)]			0.680
Non smoking	0 (0)	7 (11.29)	
~10 cigarettes	35 (62.50)	25 (40.32)	
~20 cigarettes	17 (30.36)	24 (38.71)	
>20 cigarettes	4 (7.14)	6 (9.68)	
Length of residence (years) [median (IQR)]	3 (2.86–3)	3 (3–3)	0.272
Infection, asthma, allergy in recent three months	12/56	16/62	0.577

GM: Geomatric mean; S_{logx}:standard deviation of the log-transformed variable; IQR, interquartile range. $p < 0.05$ was considered as statistically significant.

2.2. Blood sample collection and routine blood testing

A total of 2 mL venous blood was collected into an EDTA-coated plastic tube for blood pollutant evaluation and routine blood tests. Another 1 mL venous blood was collected in a plastic tube without anti-coagulants for isolation of serum. The last 1 mL of venous blood was collected in an EDTA-coated plastic tube for flow cytometry analysis. All samples were placed on ice during transportation to the laboratory. Samples for the routine blood test were used within 3 h after collection. Blood samples for pollutant evaluation were stored at −20 °C after being aliquoted in the laboratory. Serum was isolated in 6 h after sample collection and stored at −80 °C. Samples for flow cytometry analysis were processed within 10 h after collection. The routine blood test was performed on a hospital blood counting machine (Sysmex XE-2100 automated hematology analyzer, Japan) to collect information on leukocyte counts, and counts and percentages of lymphocytes.

2.3. Measurement of blood Pb levels

Blood samples were thawed from −20 °C just before use. The blood Pb level was then determined by a routine protocol used by our laboratory (Huo et al., 2007). Briefly, 100 μL of each blood sample was mixed with 900 μL of 0.5% nitric acid (AR) and digested for 10 min at room temperature. The mixture was subsequently tested by graphite furnace atomic absorption spectrophotometry (Jena Zeenit 650, Germany), with an injection volume of 20 μL, as previously described (Huo et al., 2007).

2.4. Flow cytometry analysis and absolute counts of T cell subgroups

For T cell subset analyses, 100 μL of whole blood was mixed with an optimal dilution of the following monoclonal antibodies: CD3-APC-CY7, CD4-PE, CD8-PerCP-Cy5.5, CD45RA-FITC, CCR7-PE-CF594, CD5-PE-CY7, CD27-APC, CD127-V450, CD25-BV510 (BD Bioscience). After adding all antibodies, the mixture was gently vortexed and incubated for 15 min in the dark at room temperature. Subsequently, 2 mL of 1× FACS lysing solution (BD Bioscience) was added and incubated for 10 min in the dark at room temperature. Finally, cells were washed 3 times with 200 μL washing buffer. Data was collected by a 9-colour Aria I flow cytometer (BD Bioscience) and analyzed with FACSDiva software (version 6.1.3, BD Bioscience). The following T cell subsets were determined (Mahnke et al., 2012; Schatorje et al., 2012): T cells ($CD3^+$), $CD4^+$ T cells ($CD3^+CD4^+CD8^-$), $CD8^+$ T cells ($CD3^+CD8^+CD4^-$), $CD4^+$ naïve T cells ($CD3^+CD4^+CD8^-CD45RA^+CD27^+$), $CD4^+$ central memory T cells ($CD3^+CD4^+CD8^-CD45RA^-CD27^+$, $CD4^+$ Tcm), $CD4^+$ effector memory T cells ($CD3^+CD4^+CD8^-CD45RA^-CD27^-$, $CD4^+$ Tem), $CD4^+$ terminally differentiated helper T cells ($CD3^+CD4^+CD8^-CD45RA^+CD27^-$, $CD4^+$ TteRA); $CD8^+$ naïve T cells ($CD3^+CD8^+CD4^-CD45RA^+CCR7^+CD27^+$, $CD8^+$ Tn), $CD8^+$ terminally differentiated T cells ($CD3^+CD8^+CD4^-CD45RA^+CCR7^-CD27^-$, $CD8^+$ TteRA), $CD8^+$ central memory T cells ($CD3^+CD8^+CD4^-CD45RA^-CCR7^+CD27^+$, $CD8^+$ Tcm), $CD8^+$ effector memory T cells ($CD3^+CD8^+$ $CD4^-CD45RA^-CCR7^-CD27^-$, $CD8^+$ Tem), and $CD8^+$ transitional memory T cells ($CD3^+CD8^+CD4^-CD45RA^-CCR7^-CD27^+$, $CD8^+$ Ttm). The full-minus-one (FMO) principle was adopted for gating the T cell subsets. Absolute counts of each subgroup were calculated by multiplying the total lymphocyte counts with the percentages of each subset that were determined by flow cytometry.

2.5. Measurement of serum cytokines

All cytokines and chemokines were detected using the ProcartaPlex Human Cytokine & Chemokine Panel 1A (eBioscience, USA). Beads that had been coated with either anti-human IL-15, IL-2, or IL-7 were incubated with serum samples and analyzed according to the manufacturer's instructions. Data was collected with a Luminex 200 instrument (Luminex, USA). The limit of detection for each cytokine was: IL-15 (1.1 pg/mL), IL-2 (0.8 pg/mL), and IL-7 (0.2 pg/mL).

2.6. Statistical analysis

The collected data were initially refined. A series mean method was used to supplement missing values. Normally distributed data were presented as mean ± SD, and appropriately normally distributed data were presented as the geometric mean and standard deviation of log-transformed variables ($GM \pm S_{logx}$). These data are compared by an independent sample *t*-test. Non-normally distributed data are presented as the median and interquartile range (IQR), and were compared by the Mann-Whitney *U* test (two groups) or Kruskal-Wallis H test (more than two groups). Categorical data and ratio were compared by the *chi* square test. Correlations between outcomes (percentages of T cell subsets) and covariates (child height, weight, age, gender, BMI, blood Pb levels, length of residence, parent education levels, parental smoking, average monthly family income, status of infection, asthma and allergy) were evaluated using Pearson's test (for normally distributed data) or Spearman's rank correlation coefficients (for non-normally distributed data). Multivariable-adjusted linear regression models were used to determine the association between blood Pb levels and percentages of T cell subsets. For regression analysis, non-normally distributed dependent variables were transformed using the log or square root to get an appropriately normal distribution. Covariates that were significantly associated with outcomes and that changed the effect estimates by 5% or more were included in the model. Child age and gender were included in the final model. Because there was no linear relationship between Pb levels and percentages of $CD8^+$ central memory T cells, blood Pb levels were categorized into tertiles to analyze the correlation. All analyses were performed with IBM SPSS software (version 19, IBM), and a $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Characteristics of children recruited in the study

As presented in Table 1, a total of 118 preschool children were recruited from Guiyu (e-waste exposed group, $n = 62$) and Haojiang (reference group, $n = 56$). There were no significant differences between the two groups in terms of gender, height, weight, length of residence, family daily cigarette consumption, status of infection, asthma and allergy within the recent three months prior to testing, and peripheral leukocyte counts (all $p > 0.05$). Lymphocyte counts and percentages, and average family monthly income in the exposed group were lower than in the reference group ($p < 0.05$). In contrast, age and blood Pb levels in the exposed group were higher than in reference group ($p < 0.01$). Moreover, the percentage of children with blood Pb levels >5 μg/dL (safety limit established by the United States Federal Drug Administration) in exposed group (48.39%) was also higher than that in reference group (12.50%) ($p < 0.01$).

3.2. Alteration of T cell distribution in children from Guiyu

The percentage of $CD3^+$ T cells was higher in the exposed group ($p < 0.05$, Table 2). However, there was no significant difference in absolute counts of $CD3^+$ T cells between the two groups. There was also no significant difference in the percentages and counts of $CD4^+$ T cells and $CD8^+$ T cells. In contrast, the percentage of $CD4^+$ naïve T cells ($CD4^+$ Tn) was notably lower ($p < 0.01$) whereas the percentage of CD4+ central memory T cells ($CD4^+$ Tcm) was significantly higher ($p < 0.01$) in the exposed group than in reference group. For the $CD8^+$ subsets, the percentage of $CD8^+$ central memory T cells ($CD8^+$ Tcm) was also higher in the exposed group ($p < 0.01$). No marked differences in percentages of other T cell subsets were found between two groups. These data

Table 2
Characteristics of T lymphocyte subpopulations in children.

Variables	Reference (n = 56)	Exposed (n = 62)	*p*
$CD3^+$ T cell counts ($\times 10^9$/L) (median, IQR)	2.57 (1.96–2.90)	2.39 (1.89–2.69)	0.205
$CD3^+$ T cells (%) (median, IQR)	51.40 (56.80–67.22)	65.30 (61.08–69.08)	**0.013**
$CD4^+$ T cell counts ($\times 10^9$/L) (median, IQR)	1.21 (0.99–1.48)	1.11 (0.89–1.35)	0.085
$CD4^+$ T cells (%) (mean ± SD)	50.32 ± 8.20	48.90 ± 7.31	0.323
$CD4^+$ Tn cells (%) (median, IQR)	73.00 (70.75–77.78)	70.20 (61.95–73.60)	**0.000**
$CD4^+$ Tem cells (%) (median, IQR)	3.65 (2.40–5.25)	4.30 (3.2–5.3)	0.564
$CD4^+$ Tcm cells (%) (GM ± Slogx)	21.43 ± 0.09	25.79 ± 0.09	**0.000**
$CD4^+$ T_{EMRA} cells (%) (median, IQR)	0.30 (0.20–0.675)	0.25 (0.10–0.53)	0.163
$CD8^+$ T cell counts ($\times 10^9$/L) (mean ± SD)	0.91 ± 0.39	0.91 ± 0.33	0.982
$CD8^+$ T cells (%) (mean ± SD)	35.71 ± 7.65	37.78 ± 7.13	0.130
$CD8^+$ Tn cells (%) (mean ± SD)	47.63 ± 14.83	50.80 ± 12.66	0.213
$CD8^+$ Tem cells (%) (median, IQR)	3.98 (2.18–6.41)	3.38 (2.56–5.59)	0.497
$CD8^+$ Ttm cells (%) (median, IQR)	12.72 (10.69–18.49)	14.32 (12.21–19.74)	0.080
$CD8^+$ Tcm cells (%) (median, IQR)	0.62 (0.42–1.14)	0.89 (0.56–1.47)	**0.001**
$CD8^+$ T_{EMRA} cells (%) (median, IQR)	12.60 (6.90–24.32)	11.48 (4.88–16.75)	0.176

Tn: naïve T cells; Tem: effector memory T cells; Ttm: transitional memory T cells; Tcm: central memory T cells; T_{EMRA}: terminally differentiated effector T cells. SD: stardard deviation; IQR: interquartile range. $p < 0.05$ was considered as statistically significant.

together indicated greater generation of memory T cells in children following chronic e-waste exposure.

3.3. Effect of gender and age on distribution of peripheral T cell subpopulations

Development of memory T cells is influenced by age and gender (Falcao and De-Santis, 1991; Hong et al., 2004; Lin et al., 1998; Saule et al., 2006). To explore the contribution of age and gender into the development of memory T cells, percentages of T cell subpopulations in different genders and ages were compared. As shown in Table 3, in the reference group, boys had lower percentages of total T cells ($CD3^+$) ($p < 0.01$) and $CD8^+$ central memory T cells than girls ($p < 0.01$). In contrast, there were no significant differences in percentages of all T cell subpopulations between boys and girls in the exposed group. Moreover, girls from both groups together had higher percentages of $CD8^+$ T cells, $CD4^+$ central memory T cells and $CD8^+$ central memory T cells than boys (all $p < 0.05$. respectively). With regard to age, because the number of 6-year-old children was <10, we divided all 118 children into three groups (3-year-olds, 4-year-olds, 5- to 6-year-olds). As shown in Table 4, the percentages of $CD4^+$ naïve T cells tended to be decreased and percentages of $CD4^+$ central memory T cells tended to be increased along with age, although the changes were not significant between the three groups ($p > 0.05$). In contrast, percentages of $CD8^+$ central memory T cells were initially increased (3-year-olds vs. 4-year-olds) and then decreased (4-year-olds vs. 5- to 6-year-olds) along with age ($p < 0.05$). In addition, age was negatively associated with the percentages of $CD4^+$ naïve T cells ($r = -0.028$, $p < 0.05$) whereas gender was positively associated with percentages of $CD4^+$ central memory T cells ($r = 0.205$, $p < 0.05$) and $CD8^+$ central memory T cells ($r = 0.209$, $p < 0.05$) in children (Table 5). These data together suggest that age and gender are important confounding factors for peripheral T cell distribution and may act together with environmental toxicants to alter the distribution of peripheral T cells in young children.

3.4. Association between blood Pb levels and percentages of T cell subsets

The correlation between blood Pb levels and percentages of T cell subpopulations was initially evaluated. Blood Pb levels positively correlated with the percentage of $CD4^+$ central memory T cells ($r = 0.312$, $p < 0.01$), and negatively correlated with the percentage of $CD4^+$ naïve T cells ($r = -0.317$, $p < 0.01$) (Table 5). Because there was

Table 3
Comparison of T lymphocyte subpopulations with gender in two groups.

	Reference			Exposed			Total		
	n		*p*	*n*		*p*	*n*		*p*
$CD3^+$ T cell counts ($\times 10^9$/L)			0.726			0.724			0.815
Boys	33	2.48 ± 0.92		29	2.44 (1.98–2.69)		62	2.46 (1.90–2.83)	
Girls	23	2.56 ± 0.67		33	2.25 (1.86–2.70)		56	2.40 (1.92–2.73)	
$CD3^+$ T cells (%)			**0.006**						0.081
Boys	33	59.50 (54.25–65.05)		29	65.10 (62.30–70.65)	0.573	62	62.85 (56.53–67.00)	
Girls	23	64.30 (59.90–69.50)		33	65.80 (60.95–68.60)		56	64.40 (60.75–68.78)	
$CD4^+$ T cell counts ($\times 10^9$/L)			0.380			0.893			0.597
Boys	33	1.21 ± 0.47		29	1.11 (0.92–1.37)		62	1.17 (0.94–1.42)	
Girls	23	1.32 ± 0.36		33	1.10 (0.85–1.38)		56	1.17 (0.93–1.46)	
$CD4^+$ T cells (%)			0.252						0.186
Boys	33	49.26 ± 8.00		29	48.01 ± 7.55	0.372	62	48.68 ± 7.75	
Girls	23	51.84 ± 8.43		33	49.69 ± 7.11		56	50.57 ± 7.68	
$CD4^+$ Tn cells (%)			0.429						0.091
Boys	33	73.83 ± 5.89		29	71.50 (63.20–73.80)	0.401	62	72.90 (68.40–75.56)	
Girls	23	72.53 ± 6.22		33	66.00 (60.65–73.50)		56	71.40 (63.40–74.93)	
$CD4^+$ Tcm cells (%)			0.269						**0.027**
Boys	33	21.33 ± 4.07		29	23.20 (21.70–28.20)	0.112	62	22.80 (19.40–25.15)	
Girls	23	22.60 ± 4.37		33	27.10 (22.45–32.55)		56	24.30 (21.95–28.95)	
$CD8^+$ T cell counts ($\times 10^9$/L)			0.665			0.230			0.262
Boys	33	0.93 ± 0.43		29	0.96 ± 0.37		62	0.94 ± 0.40	
Girls	23	0.88 ± 0.34		33	0.86 ± 0.29		56	0.87 ± 0.31	
$CD8^+$ T cell (%)			0.157			0.09			**0.046**
Boys	33	36.92 ± 7.82		29	39.42 ± 6.79		62	38.09 ± 7.40	
Girls	23	33.97 ± 7.21		33	36.34 ± 7.21		56	35.37 ± 7.24	
$CD8^+$ Tcm cells (%)			**0.002**			0.667			**0.024**
Boys	33	0.49 (0.40–0.84)		29	0.92 (0.55–1.32)		62	0.69 (0.46–1.14)	
Girls	23	0.84 (0.48–1.35)		33	0.87 (0.59–1.62)		56	0.84 (0.57–1.49)	

Percentages of T cell subpopulations are presented as mean ± SD (normally distributed) or median (IQR) (non-normally distributed). IQR: interquartile range. $p < 0.05$ was considered as statistically significant.

Table 4
Comparison of blood Pb levels and T lymphocyte subpopulations with different ages.

Group	Age (years)	n	$CD4^+$ T cells (%)		$CD4^+$ Tn cells (%)		$CD4^+$ Tcm cells (%)		$CD8^+$ T cells (%)		$CD8^+$ Tcm (%)	
			Mean ± SD	p	Median (IQR)	p	Median (IQR)	p	Mean ± SD	p	Median (IQR)	p
1	3–	18	49.95 ± 9.23	0.406	73.60 (70.05–78.30)	0.123	20.90 (17.73–24.43)	0.072	37.09 ± 9.18	0.607	0.53 (0.36–0.98)	0.006
2	4–	46	50.62 ± 7.90		71.85 (64.88–73.33)		23.85 (22.03–28.33)		35.92 ± 6.87		1.20 (0.70–1.47)*	
3	5–6	54	48.56 ± 7.08		71.40 (61.95–75.05)		23.20 (20.60–27.98)		37.42 ± 7.31		0.60 (0.48–1.06)**	

* $p < 0.01$, compared with group 1.
** $p < 0.01$, compared with group 2.

no linear relationship between blood Pb levels and percentage of $CD8^+$ central memory T cells, the blood Pb levels ($n = 118$) were categorized into tertiles to explore its correlation with percentages of $CD8^+$ central memory T cells. The results showed that blood Pb levels in the second tertile were positively correlated with percentages of $CD8^+$ central memory T cells ($r = 0.346$, $p < 0.01$) whereas blood Pb levels in the third tertile were negatively correlated with percentages of $CD8^+$ central memory T cells ($r = -0.554$, $p < 0.01$) (Table 5). To further explore the role of blood Pb levels on changes in the percentages of T cell subpopulations, we used a multivariable-adjusted regression model to analyze the correlation between blood Pb levels and percentages of T cell subpopulations (Table 6). After adjustment by age and gender, blood Pb levels were positively associated with the percentages of $CD4^+$ central memory T cells ($\beta = 0.487$, $p = 0.014$), and were negatively associated with the percentages of $CD4^+$ Tn cells ($\beta = -0.592$, $p = 0.015$) (Table 6). For $CD8^+$ central memory T cells, blood Pb levels in the second tertile were positively associated with the percentages of $CD8^+$ central memory T cells ($\beta = 0.028$, $p = 0.025$), and in the third tertile were negatively associated with the percentages of $CD8^+$ central memory T cells ($\beta = -0.006$, $p = 0.000$) (Table 6). No association was found between blood Pb levels in the first tertile and the percentages of $CD8^+$ central memory T cells. The regression analysis indicated that changes of blood Pb levels have a marginal contribution to alteration of the percentages of $CD8^+$ central memory T cells.

3.5. Serum levels of cytokines relevant to generation and homeostasis of memory T cells

IL-2, IL-7 and IL-15 are essential for peripheral naïve and memory T cell survival and turnover (Almeida et al., 2002; Sprent et al., 2008). Because Pb has been shown to affect cytokine production in vitro and in vivo (Heo et al., 2007; Shen et al., 2001), we expected that these T cell homeostasis-relevant cytokines mediated the association between Pb exposure and alteration of naïve and memory T cell distributions in preschool children. To test this, serum levels of IL-2, IL-7 and IL-15 were compared between the two groups (Table 7). The differences in expressions of three cytokines were not significant between the two groups, indicating that these cytokines were not the reason for the different distributions of memory T cell subpopulations in preschool children.

4. Discussion

In this study, we found that children from Guiyu, an e-waste-contaminated area, have higher blood Pb levels and increased percentages of $CD4^+$ and $CD8^+$ central memory T cells compared with children from Haojiang, a reference area. In addition, blood Pb levels were positively associated with the percentage of $CD4^+$ central memory T cells. The present study suggests that Pb exposure might contribute to increased percentages of $CD4^+$ central memory T cells in children from an e-waste-contaminated area.

In the current study, blood Pb levels are positively associated with percentages of $CD4^+$ central memory T cells, but negatively associated with percentages of $CD4^+$ naïve T cells. The positive association between blood Pb levels and $CD4^+$ central memory T cells is still strong after including age and gender in regression model, suggesting that Pb exposure may promote $CD4^+$ memory T cell development in children. Previous studies demonstrated that development of memory T cells in a primary immune response is determined by the strength of T cell receptor (TCR) signaling, initiated by antigen binding, and length of interaction between TCR and antigen (Kim et al., 2013; Williams et al., 2008). Pb might also interact with TCR signaling pathway to promote or suppress memory T cell development. Interestingly, an in vitro study showed that low levels of Pb (2 μg/dL) can promote proliferation of $CD4^+$ T cells under alloantigen stimulation, but do not enhance the proliferation of $CD4^+$ T cells following stimulation by mitogens or super antigens (without antigen presentation), suggesting that Pb might affect antigen processing/presentation process to alter $CD4^+$ T cell proliferation (McCabe et al., 2001). Further study demonstrated that low levels of Pb target $CD11c^+$-antigen presenting cells and might modulate specific peptide:MHC interactions to enhance $CD4^+$ T cell proliferation (Farrer et al., 2005). In addition, Pb is found to enhance interaction between antigen-specific T cells and B cells and promote T helper cell 2 (Th2) rather than Th1 activation (McCabe and Lawrence, 1991). In current study, Pb exposure might have modified naïve $CD4^+$

Table 5
Spearman's correlation analysis between covariates and T lymphocyte subpopulations.

	Age ($n = 118$)		Gender ($n = 118$)		Blood Pb levels ($n = 118$)	
	r	p	r	p	r	p
$CD4^+$ Tn (%)	−0.202	**0.028**	−0.156	0.091	−0.317	**0.000**
$CD4^+$ Tcm (%)	0.142	0.124	0.205	**0.026**	0.312	**0.001**
$CD8^+$ Tcm (%)	−0.124	0.183	0.209	**0.023**	0.121	0.191

$p < 0.05$ was considered as statistically significant.

Tertiles of blood Pb levels (μg/dL)

	1.490–3.526 (n = 29)		3.531–5.044 ($n = 60$)		5.144–16.24 (n = 29)	
	r	p	r	p	r	p
$CD8^+$ Tcm (%)	0.181	0.347	0.346	**0.007**	−0.554	**0.002**

$p < 0.05$ was considered as statistically significant. First tertile: 1.490−3.526 μg/dL; Second tertile: 3.531−5.044 μg/dL; Third tertile: 5.144−16.24 μg/dL.

Table 6
Multivariate linear regression analysis of effect of blood Pb levels on T lymphocyte subpopulations.

Blood Pb levels ($n = 118$)		
	Unstardardized β	*P*
$CD4^+$ Tn (%)	−0.592	0.015
$CD4^+$ Tcm (%)	0.487	0.014

Data was adjusted by age, gender. $p < 0.05$ was considered as statistically significant

Tertiles of blood Pb levels (μg/dL)						
	1.490–3.526 ($n = 29$)		3.531–5.044 ($n = 60$)		5.144–16.24 ($n = 29$)	
	Unstandardized β	*p*	Unstandardized β	*p*	Unstandardized β	*p*
$CD8^+$ Tcm (%)*	–	–	0.028	0.025	−0.006	0.000

*: square root transform. Data was adjusted by age, gender. $p < 0.05$ was considered as statistically significant.

T cell responses and finally increase percentages of $CD4^+$ memory T cells in Guiyu children. It is argued that Guiyu children have a greater frequency of infections or suffer from higher frequencies of asthma, allergy or smoking exposure, which result in the increase of memory T cell development. However, there was no significant difference in frequencies of these confounding factors between Guiyu and Haojiang children. It is also argued that higher levels of cytokines, such as IL-2, IL-7, or IL-15 are the reasons for the increased $CD4^+$ memory T cells in Guiyu children. Indeed, these cytokines are not only important for memory T cell generation, but also for maintenance of memory T cells in the periphery (Bradley et al., 2005; Marsden et al., 2006; Prlic et al., 2002). However, there were no marked differences in levels of the serum cytokines between Guiyu and Haojiang children. These results also indicate that Pb does not affect development of memory T cells through altering expression of these cytokines, although it can modulate production of several cytokines from T cells. Together, current study indicates that Pb exposure promotes $CD4^+$ memory T cells in Guiyu children.

The observation that no difference in counts of $CD3^+$ T cells, counts and percentages of $CD4^+$ and $CD8^+$ T cells between reference group and exposed group in present study indicates that Pb exposure did not cause major T cell death in e-waste-exposed children, which is different from previous results. Previous studies showed that higher blood Pb levels are associated with reduced percentages and counts of $CD3^+$ T cells and $CD4^+$ T cells in occupationally Pb-exposed workers and elder children (Coelho et al., 2014; Fischbein et al., 1993; Garcia-Leston et al., 2012; Undeger et al., 1996). Moreover, counts of $CD3^+CD45RO^+$ memory T cells in Pb-exposed adult workers are decreased, and the blood Pb levels (19 μg/dL, mean) are even positively associated with expansion of $CD8^+$ T cells (Sata et al., 1998). The discrepancy between previous studies and the current study emphasizes the importance of levels of Pb exposure in evaluation of Pb-induced toxicity to T cells. Indeed, blood Pb levels in Guiyu children (5.06 ± 0.17 μg/dL) in the present study is much lower than that in previous studies. Other factors, such as age and other e-waste contiminants may also be the reason for the discrepancy. Pb exposure may act independently or synergistically with these factors to influence T cell responses and memory T cell development in children, and cause discrepant outcomes. Of note, a higher percentage of $CD3^+$ T cells was observed in the Pb-exposed group. However, counts of $CD3^+$ T cells did not undergo a marked change when compared to the reference group. The lower lymphocyte counts of in present study may account for the increased percentages of $CD3^+$ T cells. Indeed, lower counts and percentages of NK cells were observed in Pb-exposed children in our previous studies (Zhang et al., 2016; Zhang et al., 2017). The previous and present studies in our group indicate that T cells may be not be as sensitive to Pb toxicity as other lymphocytes, such as NK cells, in preschool children. However, it is difficult to determine the cut-off value of blood Pb levels that could promote or suppress development of $CD4^+$ central memory T cells in the current study, because blood Pb levels in most of the studied population were <10 μg/dL (111 in total 118 samples) and correlation analysis showed a positive relationship between blood Pb levels and percentages of $CD4^+$ central memory T cells. Further studies that include larger sample size and higher blood Pb levels would help to fix the question. Together, our data suggests that blood Pb levels within a certain range may facilitate $CD4^+$ memory T cell development rather than threaten T cell survival.

In the present study, the percentage of $CD8^+$ central memory T cells were positively associated with blood Pb levels in the second tertile, negatively associated with blood Pb levels in the third tertile. However, linear regression analysis showed that changes of blood Pb levels had a significant, but very marginal contribution to the alteration of the percentages of $CD8^+$ central memory T cells (Table 6), indicating that factors, other than Pb, may affect development of $CD8^+$ central memory T cells in children. Moreover, percentages of $CD8^+$ central memory T cells are initially increased (3-year- olds vs. 4-year-olds). However, they are decreased in 5- to 6-year-old children when compared to 4-year-old children, which is in contrast to previous studies (Falcao and De-Santis, 1991; Hong et al., 2004; Lin et al., 1998; Saule et al., 2006). It is possible that children at younger ages may have less exposure to Pb and other environmental toxicants, low levels of Pb and other toxicants may stimulate development of $CD8^+$ central memory T cells. However, children at elder ages may have greater exposure to Pb and other environmental toxicants, which might suppress development of $CD8^+$ central memory T cells.

In healthy adults, females usually have a higher $CD4^+$ T cells counts than males (Apoil et al., 2017; Uppal et al., 2003). However, we do not observe marked differences in percentages of $CD4^+$ T cells between boys and girls in either the reference group or exposed group, or after combining the two groups in present study. In contrast, girls have a lower percentage of $CD8^+$ T cells than boys in the combined group. A possible explanation for the discordance is that disuniformed developmental dynamics of $CD4^+$ and $CD8^+$ T cells exist in young male and female children and the difference in counts of $CD4^+$ T cells does not become significant until they reach adulthood. Notably, we observed that female children from the combined group have higher percentages of both $CD4^+$ and $CD8^+$ central memory T cells than male children, and gender is correlated with percentages of $CD4^+$ central memory T cells and $CD8^+$ central memory T cells (Table 5). In linear regression analysis, gender and blood Pb levels contributed almost equally in increased percentages of $CD4^+$ central memory T cells (standardized β: 0.236 vs. 0.228, gender vs. blood Pb levels, data not shown), suggesting an essential role of gender on $CD4^+$ central memory T cell development. Our results emphasize the importance of gender in exploring the effect of Pb exposure on development of memory T cells in children. Sex steroid hormones, such as estradiol testosterone, have been suggested to mediate gender effect on T cell immune response (Giefing-Kröll et al., 2015). Whether Pb exposure alters levels of sex steroid hormones and subsequently affects on development of $CD4^+$ memory T cell in children needs further investigation.

Table 7
Cytokines relevant to memory T cell generation and homeo-stasis in children.

	Reference (pg/mL)	Exposed (pg/mL)	*p*
	Median (IQR)	Median (IQR)	
IL-2	8.41 (5.25–12.09)	11.04 (4.13–11.61)	0.786
IL-7	6.82 (5.39–8.20)	6.71 (5.28–7.77)	0.478
IL-15	21.95 (15.52–30.59)	27.27 (18.44–30.62)	0.130

$p < 0.05$ was considered as statistically significant.

There are several limits in the current study. First, there were no marked correlations between some confounding factors (such as family smoking status, infections and allergic diseases) and percentages of memory T cells, although they are important in influencing memory T cell development. A possible reason is that the sample size in our study is not large enough to set up a statistically significant correlation. Second, we were not able to assess the status of microbial exposure in children, or hospital visitation records for infections or allergic diseases, which are also crucial for memory T cell development. Third, only the effect of Pb exposure on the development of memory T cell was evaluated in present study due to limitations of blood volume. Other heavy metals, such as mercury, cadmium and organic toxicants might also be present in the children's blood and affect memory T cell development. These limits may result in an under-estimation or over-estimation of the role of Pb exposure on development of memory T cells. However, the present study provides preliminary evidence that Pb exposure within certain ranges may contribute to the increased percentages of $CD4^+$ central memory T cells in young children.

In conclusion, we found higher percentages of peripheral $CD4^+$ and $CD8^+$ central memory T cells in children from an e-waste recycling area. Pb exposure might contribute to the increased percentages of peripheral $CD4^+$ central memory T cells. The present study brings to light a concern that Pb exposure could promote a biased development of $CD4^+$ memory T cells in children. Longitudinal studies are needed to corroborate current observations, and to test the performance of memory T cells in these Pb-exposed children.

Conflict of interest

All authors declared no conflict of interest.

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