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Effect of lead exposure on the immune function of lymphocytes and erythrocytes in preschool children*

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Abstract: Objective: To investigate the influence of lead exposure on the immune function of lymphocytes and erythrocytes in preschool children. Materials and methods: A group of 217 children three to six years of age from a rural area were given a thorough physical examination and the concentration of lead in blood samples taken from each subject was determined. The indices of lymphocyte immunity ($CD^+_3CD^+_4$, $CD^+_3CD^+_8$, $CD^+_4CD^+_8$, $CD^-_3CD^+_{19}$) and erythrocyte immunity (RBC-C_3b, RBC-IC, RFER, RFIR, CD_{35} and its average fluorescence intensity) of 40 children with blood lead levels above 0.483 μmol/L were measured and compared with a control group. Results: The blood lead levels of the 217 children ranged from 0.11 μmol/L to 2.11 μmol/L. The $CD^+_3CD^+_4$and $CD^+_4CD^+_8$ cells were lower (P<0.01) and the $CD^+_3CD^+_8$ cells were higher in the lead-poisoned subjects than those in the control group (P<0.05). CD^+_3 and $CD^-_3CD^+_{19}$ did not show significant differences. Although the RBC-C_3b rosette forming rate was lower and the RBC-IC rosette forming rate was higher in the lead-poisoned group, this difference could not be shown to be statistically significant (P>0.05). RFIR was found to be lower in the lead-poisoned group (P<0.01). Compared with the control group, the positive rate of CD_{35} was not found to be significantly different in a group of 25 lead-poisoned children (P>0.05), while the average fluorescence intensity was lower in the lead-poisoned group (P<0.05). Conclusion: Lead exposure can result in impaired immune function of T lymphocytes and erythrocytes in preschool children.

Key words: Children, Lead exposure, Lymphocyte immunity, Erythrocyte immunity
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INTRODUCTION

Over the past few years, systematic studies on the effect of lead exposure on the human body have led to an understanding that lead is neurotoxic and has adverse effect on the immune system. So far, however, research on the immunotoxic effects of lead has been limited to animal experiments and people with occupational exposure to lead (Shen *et al*., 1996; Mishra *et al*., 2003; Liu *et al*., 2000). Presently, there are no reports on the influence of lead exposure on lymphocyte and erythrocyte immunity in children, especially of preschool age.

In this study, markers of lymphocyte immunity were measured in 38 children with lead blood level ≥0.483 μmol/L, and markers of erythrocyte immunity were measured in 40 children with lead blood level ≥0.483 μmol/L. In 25 subjects from the

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latter group, the rate of CD_{35} positive cells and the average fluorescence intensity were determined.

MATERIALS AND METHODS

Two hundred seventeen (96 boys, 121 girls) preschool children 3 to 6 years of age were selected among children of permanent residents in a rural area in Zhejiang Province. Laboratory check of blood samples revealed lead blood levels ≥0.483 μmol/L in 63 cases assigned to the lead-poisoned group, while a corresponding number of cases were randomly selected for the control group.

The body height and the weight of the children were measured and they were given a thorough physical examination. The cervical skin was repeatedly cleaned of lead with 0.1% EDTA and ion-free water and disinfected with tincture of iodine. Then 2.0 ml of venous blood samples were taken and anticoagulated with 0.5 ml 7% EDTA. Blood levels of lead were determined using an atomic absorptiospectrometer AA700 manufactured by Perkin Elmer Company (USA) and the graphite oven atom absorption spectrography method. The blood lead control reagents were provided by the Wisconsin State Hygiene Laboratory (USA).

For the determination of lymphocyte immunity, 1.5 ml of anticoagulated venous blood were used for fluorescence antibody staining within 24 hours. The sample in each of the three tubes was simultaneously stained. The sample in the third tube served as the control for the test of the four markers. Each of the 3 tubes contained 10 μl of fluorescence antibody and 50 μl of anticoagulated blood. After thorough mixing, the tubes were incubated in refrigerator at 4 °C for 30 minutes. Then 1 ml hemolysis reagent FASS (B-D Company, USA) was added, the content of the tubes was automatically mixed at high speed, followed by another incubation for 10 minutes in the dark at room temperature. The tubes were then centrifuged at 400×g, and left for sedimentation for 5 minutes; after which the supernatant was removed, and the sediment was thoroughly mixed, washed in 2 ml PBS, and finally stored in refrigerator at 2~8 °C. Analysis by flowcytometre (B-D Company, USA) was completed within 24 to 48 hours after the staining.

For the determination of lymphocyte immunity, 0.5 ml blood was anticoagulated with heparin. The RBC-C3b rosette forming rate and the RBC-IC rosette forming rate were determined by RBC-C_3b rosette forming and RCB-IC rosette forming assay. RFED and RFIR were determined in 0.5 ml serum by erythrocyte immunomodulating factors activity assay. The following reagents were used: complement activated zymosan lyophilised powder, zymosan lyophilised powder (Shanghai Hospital, Laboratory of Immunology, Shanghai) and normal human erythrocyte suspension (blood type O). A flowcytometre (B-D Company, USA) was used to measure the rate of CD35 positive cells and the average fluorescence intensity.

All statistical analyses were performed using the computer program Statistical Package for the Social Sciences (SPSS) 10.0. Differences between groups for continuous data were analyzed with independent-samples *t* test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Blood lead levels of 217 preschool children

The blood lead levels measured ranged from 0.11 μmol/L to 2.11 μmol/L. Sixty-three subjects (29.3%) with blood lead levels ≥0.483 μmol/L were identified. In that group, the difference in the blood lead level between boys (0.489±0.29 μmol/L) and girls (0.487±0.25 μmol/L) was not statistically significant.

Effect of different blood lead levels on lymphocyte immunity in preschool children

The applied selection standard excluded from the study children who had had vaccinations, respiratory tract infections or had taken immunomodulating drugs shortly before the examination. Subsequently, thirty-eight children were assigned to the lead-poisoned group, while a sample of 35 random-

ly selected subjects with blood lead level <0.483 μmol/L were assigned to the low-lead control group. Comparison of the results of the test and the control group showed that the $CD^+_3CD^+_4$ and the $CD^+_4CD^+_8$ cells were lower (P<0.01) and the $CD^+_3CD^+_8$ cells were higher in the lead-poisoned group (P<0.05). And that the CD^+_3 and the $CD^-_3CD^+_{19}$ did not show significant differences (P>0.05) (Table 1).

Effect of different blood lead levels on the erythrocyte immunity in preschool children

Based on the applied selection criteria, 40 children with blood lead level ≥0.483 μmol/L were assigned to the lead-poisoned group, while the control group comprised 35 subjects. The RBC-C_3b rosette forming rate was reduced and the RBC-IC rosette forming rate was increased in the lead-poisoned group. This difference, however, did not show statistical significance (P>0.05). RFIR was significantly lower in the lead-poisoned group (t=3.370, P<0.01) (Table 2).

Effect of different lead blood levels on the rate of CD_{35} positive cells and the average fluorescence intensity in preschool children

Based on the applied selection standard, 25 children with blood lead level ≥0.483 μmol/L were assigned to the lead-poisoned group, while the control group included 21 subjects (Table 3).

DISCUSSION

Lead is one of the most commonly encountered toxic elements. An increasing number of studies proved that even low doses of lead could lead to toxic effects in children exposed to it. Lead is suspected to be a causal factor for two potential immunotoxic effects. Past research on the immunotoxic effects of lead was limited to animal experiments and persons with occupational exposure to lead. Up to now, there are no reports on how lead affects the immune system in children, or preschool children in particular.

On the basis of measuring the blood lead level, our study identified 63 preschool age children with lead-poisoning; 38 of whom met the applied selection criteria and were included in the experimental group for determining lymphocyte immunity indices. We found levels of the $CD^+_3CD^+_4$ and the $CD^+_4CD^+_8$ positive cells to be significantly reduced, while the $CD^+_3CD^+_8$ cells significantly increased in the lead-poisoned group at lead blood level ≥0.483 μmol/L. This shows that certain subgroups of T lymphocytes in preschool children are extremely sensitive; that long-term low-level lead exposure may impair the ability of the TCR-CD_3 compound to differentiate between antigens and may also influence the signal transmission process of the cell activation. That poses a great threat to the not yet

Table 1 Comparison of lymphocyte immunity between two groups ($\bar{x}\pm SD$) in %

	Group		t-value
	Control	Lead-poison	
Cases	35	38	
CD^+_3	55.20±6.77	54.61±8.26	0.334
$CD^+_3CD^+_4$	27.10±5.83	23.68±4.81	2.377**
$CD^+_3CD^+_8$	20.57±4.84	23.21±5.77	2.110*
$CD^+_4CD^+_8$	1.41±0.50	1.09±0.37	3.120**
$CD^-_3CD^+_{19}$	16.58±4.60	16.82±6.64	1.089

*P<0.05, **P<0.01

Table 2 Comparison of erythrocyte immunity between two groups ($\bar{x}\pm SD$) in %

	Group		t-value
	Control	Lead-poison	
Cases	35	40	
RBC-C_3b	19.33±3.97	18.83±3.33	0.595
RBC-IC	5.91±2.72	6.68±2.70	1.202
RFER	87.10±30.94	83.57±37.15	0.435
RFIR	74.27±15.50	58.61±22.66	3.370**

*P<0.01

Table 3 Rate of CD_{35} Positive findings and average fluorescence intensity in two groups ($\bar{x}\pm SD$)

	Group		t-value
	Control	Lead-poison	
Cases	21	25	
Rate of CD_{35} positive findings (%)	30.39±12.10	23.64±13.53	1.767
Average fluorescence intensity	4.92±2.97	3.24±2.52	2.085*

*P<0.05

fully matured lymphocytes immune system in children (Fichbein *et al*., 1993; Stata and Araki, 1997; McCabe *et al*., 2001).

On the other hand, it was observed that $CD_3^-CD_{19}^+$ did not show significant differences in the lead poisoned group as compared with the control group. This result indicated that low level exposure to lead might not significantly affect the level of peripheral B lymphocyte. Earlier studies also indicated that T cells are believed to be critical functional targets of lead immunotoxicity (Stata and Araki, 1997). The influence of low level lead exposure on B lymphocyte immune functions in children needs further study.

Erythrocytes have an important respiratory function and also play an important role in immunity. They can differentiate between, adhere to, and concentrate antigens, and accelerate the CIC function, and thus take part in the immune modulation of the body. CRI (CD_{35}) serves as the basis for the immunologic functions of erythrocytes. CD_{35} can be identified on the cell membrane of many different cell types, with 95% of all the CD_{35} in the blood circulation system being found on the cell membrane of erythrocytes. After having been resorbed from the GI tract, lead enters the blood circulation and meets the red blood cells. More than 99% of the lead present in the circulatory system is bound to the erythrocytes, 80% of which is located in the cytoplasm and 20% of them being found on the cell membrane. In children affected by lead poison, a change in CD_{35} can be expected to reflect the effect of the lead exposure on the erythrocyte-mediated immunity. In this study, significant difference was found for the CD_{35} average fluorescence intensity ($P<0.05$). The rate of CD_{35} positive cells also revealed a tendency of decline in the experimental group, even though this difference could not be shown to have statistical significance in the experimental group ($t=1.767$, $P=0.084$). This might be a result of the small number of subjects in this study.

Earlier investigations (Wang *et al*., 1994; Gao *et al*., 1995) showed that erythrocyte immunity and erythrocyte immune factors undergo changes in the presence of lead poisoning during which the rosette forming rate of RBC-C_3b receptors decreases, and the rosette forming rate of RBC-IC increases, RFER decreases and RFIR increases. The results of our study showed a decrease in the rosette forming rate of RBC-C_3b receptors and an increase in the rosette forming rate of RBC-IC. Comparison with the control group, however, did not show statistical significance ($P>0.05$). Still, significantly decreased levels of RFIR were found ($P<0.01$). The blood lead levels in our study ranged from 10.0 to 19.0 μg/dl, implying a light degree of lead poisoning. With more severe poisoning and a subsequent further increase in erythrocyte adhesion factors and inhibiting factors, it may be possible that the changes in the rosette forming rate of RBC-C_3b receptors and RBC-IC may reach a statistically significant degree. This hypothesis will be subject to further investigations.

References

Fischbein, A., Tsang, P., Luo, J.C., Roboz, J.P., Jiang, J.D., Bekesi, J.G., 1993. Phenotypic aberration of CD_3 and CD_4 cell and functional impairments of lymphocytes at low-lead occupational exposure to lead. *Clin Immunol Immunpathol*, **66**(2):163-168.

Gao, G.W., Zhu, A.X., Tang, Y.H., 1995. Pilot study on erythrocyte immunity in humans with lead poisoning. *Journal of Chinese Workers Hygiene and Occupational Disease*, **13**(1):28 (in Chinese).

Liu, Q., Chen, R., Qin, R., 2000. Effect of sub-acute low level exposure to lead on cellular immune function in rats. *Wei Sheng Yan Jiu*, **29**(6):354-356 (in Chinese).

McCabe, M.J. Jr., Singh, K.P., Reiners, J.J. Jr., 2001. Low level lead exposure in vitro stimulates the proliferation and expansion of alloantigen-reactive CD_4 (high) *T* cells. *Toxicol Appl Pharmacol*, **177***(*3):219-231.

Mishra, K.P., Singh, V.K., Rani, R., 2003. Effect of lead exposure on the immune response of some occupationally exposed individuals. *Toxicology*, **188**(2-3): 251-259.

Shen, X.M., Yan, C.H., Ao, L.M., Zhao, W., Lin, J.S., 1996. Lead Poisoning in Children. First edition, Beijing People's Hygiene Publishing Company, Beijing (in Chinese).

Stata, F., Araki, S., 1997. Changes in natural Killer cell subpopulations in lead workers. *Int Arch Occup Environ Health*, **69**(5):305.

Wang, G.D., Huang, F.R., Lin, R.C., 1994. Exploration of the effects of lead on the human erythrocyte immunity functions. *Industrial Hygiene and Occupational Disease*, **20**(1):45-47 (in Chinese).