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ORIGINAL ARTICLE

Alteration of Th1/Th2/Th17 cytokine profile and humoral immune responses associated with chromate exposure

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ABSTRACT

Background The role of chromate exposure in the deregulation of total lymphocyte and other immune factors is largely unclear.

Objectives We aimed to examine alteration of the Th1/Th2/Th17 cytokine profile and humoral indicators caused by occupational chromate exposure.

Methods A cross-sectional study was conducted in two similar workshops (groups 1 and 2) with 106 male occupational workers and 50 matched local controls. Environmental and biological exposures were assessed by measuring chromium concentrations in workplace air, and in whole blood and urine samples of the workers. Cytokines in serum (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , IL-17A) were determined by CBA assay, while immunoglobulin (IgA, IgM, IgG, IgE) and complement (C3, C4) were evaluated by immunonephelometric and ELISA methods. Micronucleus analysis was also used to explore the relationship between genotoxicity and immunotoxicity.

Results Compared with the control group, environmental chromate exposure in groups 1 and 2 was much higher, and the mean values of IL-6, IL-10, IFN- γ , IL-17A and IFN- γ /IL-4 were significantly decreased in group 1. In group 2, IgA and IgG levels were reduced, while C3 and C4 were increased. Levels of IFN- γ , IgG and IgA were all inversely associated with whole blood chromium, while C3 and C4 were positively associated with whole blood chromium ($p < 0.05$). Both IL-10 and IL-17A were inversely associated with urine chromium. Correlations were also found between IL-10, IL-17A and micronucleus ($r = -0.329$, $r = -0.312$, respectively).

Conclusions Occupational exposure to chromate could downregulate the cellular and humoral factors of the immune system.

INTRODUCTION

Chromium exposure is a common risk factor for occupational workers as it is found in chrome-plated metal, leather, pigments, etc. Moreover, it is a well known occupational carcinogen in the chrome-plating, stainless steel and pigment industries, and can directly affect human health especially in the form hexavalent chromium (Cr-VI). Cr-VI mainly enters the human body through inhalation during occupational exposure. Once in the respiratory system (nose, bronchi and lung), some Cr-VI may be converted to Cr-III and accumulate in the broncho-alveolar lining fluid, mucosa and pulmonary tissues.^{1 2} This process can produce many reactive intermediates with

What this paper adds

- Previous studies have found that chromate exposure can affect the immune system, but there has been little investigation of the impact on total lymphocyte deregulation and other immune factors.
- Our results demonstrate that chromate exposure can produce effects on lymphocytes, including inhibition of cytokine secretion and immunoglobulin production, and activation of complement generation.
- These findings show that immune indicators can be used as early effective biomarkers of chromate exposure in occupational health monitoring.

consequent oxidative stress, which can contribute to chromate cytotoxicity, genotoxicity and carcinogenicity.³ Some epidemiological studies have suggested that immunotoxicity may be caused in this situation.^{3 4} When chromium is transported by erythrocytes and lymphocytes in the blood, it can interact with various proteins, DNA and other biological macromolecules, which can threaten the health of the human body.^{5 6}

The immune system is an important and complex system composed of different types of cells and factors with various functions, upon which the health of the human body depends. Persistent immune dysfunction can cause many chronic diseases including cancer. In addition, this system is one of the most sensitive targets for environmental pollutants or hazards and is regulated by many immune factors.⁷⁻⁹ Cytokines in this system are important immune response modulators but can be affected by metal exposure.¹⁰ Most cytokines are secreted by T helper (Th) cells and their measurement can provide feedback on differentiation regulation.¹¹ When the human body is affected by harmful occupational or environmental substances, the cytokine balance is disturbed, possibly leading to disease. Similar effects are also caused by antibodies/immunoglobulin (Ig) and complement (C). The balanced interplay of various immune cells, such as lymphocytes, T cells, B cells, NK cells and monocytes, and the regulation of cells through the production of immune factors is essential for human health.

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Previous studies have found that Cr-VI plays an important role in altering the immune response by immunostimulatory or immunosuppressive processes which were shown by its effects on T and B lymphocytes, macrophages, cytokine production and the immune response, possibly inducing hypersensitivity reactions.^{12–13} Researchers have found that the levels of different immune factors in occupationally exposed workers could be altered,^{14–16} which could affect the function of the immune system thus endangering the health of workers.

However, the few studies on immunological alterations caused by chromium show conflicting results.^{17–18} Therefore our research aimed to observe the effect of chromate on immune status, especially on cytokine production and immunoglobulin and complement secretion through chromate exposure level assessment. We examined Th1/Th2/Th17 cytokine expression for cellular immunity alteration, and immunoglobulin and complement expression as indicators of humoral immunity alteration in chromate exposed workers in order to determine the effect of occupational chromate exposure on immunotoxicity.

MATERIALS AND METHODS

Study design and population

This was a cross-sectional study. The factory chosen for the study was selected as (1) it only produced potassium dichromate, a water-soluble hexavalent chromium compound; and (2) it offered annual health check-ups for chromate production workers, which allowed us to easily collect specimens from workers during their annual physical examinations to minimise interference with normal work schedules.

Workers in two similar workshops in the factory were selected as the exposed groups (group 1 and 2), so that the accuracy of the measurements for chromium compounds could be compared. The workers carried out the same activities and were exposed to the same occupational hazards. A total of 106 male workers exposed to chromium in the two workshops were enrolled in the study: the 50 workers in group 1 were used to examine chromate's effect on cytokine production, while the 56 workers in group 2 were chosen for analysis of humoral immunity. The controls were 50 non-exposed individuals living approximately 20 km from the factory. The criteria for inclusion included: (1) age 25–50 years; (2) no medical history of allergy, asthma or allergic rhinitis; and (3) no skin infections, fever or other clinical diseases. Workers also had to have at least 1 year of employment in the factory and 3 months in the same work location.

All subjects were blue-collar workers or farmers with a similar education, social background and geographical location. All underwent a clinical examination and completed a questionnaire enquiring about their occupational history, personal medical history, medication used in the 4 weeks before the study, body weight and height, hair dye use, house decoration, radiation exposure, individual protection, smoking status and alcohol intake.

This study was approved by the Medical Ethics Committee of Peking University, Health Science Center (HSC), Beijing, China. Written informed consent from each study subject was also obtained.

Air and biological exposure assessment

The potassium dichromate produced in the factory is a water-soluble hexavalent chromium compound. In accordance with the sampling criteria for monitoring hazardous substances in air (GBZ 159–2004), we took samples from six representative areas in each workshop. Air is sampled at 10 sampling points in each area by pumps working at a rate of 1 l/min for 8 h (Sp730; TSI, Shoreview, Minnesota, USA) and fitted with MCE mixed

cellulose ester filters (37 mm diameter; Pall, Ann Arbor, Michigan, USA). After sampling, the concentration of chromium on the membranes was measured by atomic absorption spectrometry; the detection limit for chromium in air was 0.001 µg/l.

All blood samples from workers exposed to chromate were collected at the end of five consecutive working days, and from the control group after completion of the questionnaire.

A 10 ml sample of peripheral blood was taken from each subject and divided between tubes containing pro-coagulant and anticoagulant for the measurement of whole blood chromium (WB-Cr). The background values of all tubes were checked beforehand to ensure the experimental results were not contaminated. Coagulant blood samples were centrifuged within 1 h. Serum was cooled to –20°C, and then stored within 6 h at –80°C until use.

A 50 ml urine sample was collected from all subjects, transferred to a centrifuge tube and then to a freezing tube for chromium (U-Cr) and creatinine determination; creatinine concentrations were adjusted to maintain the same U-Cr to urine ratio in all samples.

Both WB-Cr and U-Cr were measured by an inductively coupled plasma mass spectrometer (ICP-MS). Creatinine was evaluated according to WS/T 9721997. The detailed procedure has been described previously (Sarmiento-González A *et al* 2008; Song YS *et al* 2012). The detection limits for WB-Cr and U-Cr were 0.0012 and 0.03 µg/l, respectively.

Determination of serum immune factors

Serum cytokines including IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17A were determined by cytometric bead array (CBA) flow cytometric assay (Human Th1/Th2/Th17 Cytokine Kit; BD Biosciences, Franklin Lakes, New Jersey, USA) according to the manufacturer's instructions.

Data were acquired on a BD FACSCaliber flow cytometer for the negative control in each assay. Standard curves were constructed and calculations performed using BD Biosciences CBA software. The standard curves were acquired with R^2 at 0.9994 for IL-2, 0.9995 for IL-4, 0.9989 for IL-6, 0.9992 for IL-10, 0.9991 for TNF-α, 0.9965 for IFN-γ and 0.9965 for IL-17A. If the fluorescent signal of a sample was equal to or below that in the control group, the cytokine level was set as zero.

The levels of serum immunoglobulin (IgA, IgM and IgG) and complement (C3 and C4) were evaluated by immunonephelometric assay⁴⁴ using an Olympus AU5400 automatic analyser (Olympus, Tokyo, Japan) with an immunonephelometric assay kit (Jiu Qiang Diagnostic Products, Beijing, China). Concentrations of serum IgE were determined by isotype specific enzyme-linked immunosorbent assays (ELISA).⁴³

Micronucleus experiments

A 0.4 ml aliquot of heparinised peripheral blood from subjects in group 1 and the control group was used for micronucleus experiments⁶ to measure genetic damage in order to study the relationship between chromate exposure and cellular immunotoxicity. The sample was added to 5 ml of RPMI-1640 culture medium and cultures were incubated at 37°C for 72 h. After 44 h of incubation, 200 µl (6 µg/ml) of cytochalasin-B (Sigma-Aldrich, St Louis, Missouri, USA) was added to the cultures and cells were harvested. The numbers of micronuclei were counted in 1000 binuclear lymphocytes of each individual according to Fenech's protocol.⁴²

Statistical analysis

EpiData software was used to enter the experimental data into computers. Double-entry and logistical error checks were used to ensure accuracy.

All analysis was performed with SPSS 16.0. Differences in continuous and categorical parameters between exposed and non-exposed subjects were tested using the two sample t test (or Mann–Whitney U test) and the χ^2 test. Linear regression analyses were carried out, with each model including one corresponding exposure variable and one outcome variable. Normality was assessed by the Kolmogorov–Smirnov test, and Pearson's and Spearman's correlations were performed. Statistical significance for two-sided p values was defined as $p < 0.05$.

RESULTS

Descriptive statistics

The exposed group consisted of 106 male workers with 1 year or more of chromate exposure. Personal protective equipment (including protective gloves and masks) was worn by more than 90% of subjects in each workshop. There were 50 matched controls. Compared to the control group, more workers in group 1 smoked and consumed alcohol ($p < 0.05$). A stratified analysis of cytokine levels showed that the subjects in group 2 had a similar distribution (see online supplementary table 3). The demographic characteristics of the study population are presented in table 1.

Figure 1 shows that air chromium concentrations were much higher for group 1 ($14.38 \pm 18.08 \mu\text{g}/\text{m}^3$) and group 2 ($28.55 \pm 29.70 \mu\text{g}/\text{m}^3$) than the control group (close to zero) ($p < 0.01$). Chromium levels in all groups were both under the water soluble exposure limit of $50 \mu\text{g}/\text{m}^3$ (ACGIH, 2012). Levels of WB-Cr and normalised U-Cr in groups 1 and 2 were all significantly higher than in the control group ($p < 0.001$, Mann–Whitney non-parametric U test). Details are given in table 1.

Levels of cytokines, immunoglobulin and complement in serum

The levels of seven cytokines were measured and the results are given in table 2. Four cytokines (IL-6, IL-10, IFN- γ , IL-17A) were down-regulated in the serum of group 1 compared to the control group ($p < 0.05$), while the concentrations of IFN- γ and IL-4 were increased in group 1 ($p < 0.05$).

The immunoglobulin and complement levels in chromate-exposed workers in group 2 and control group subjects were determined to assess overall immunological changes. IgG and IgA levels were reduced, and C3 and C4 levels were increased in group 2 compared with the control group.

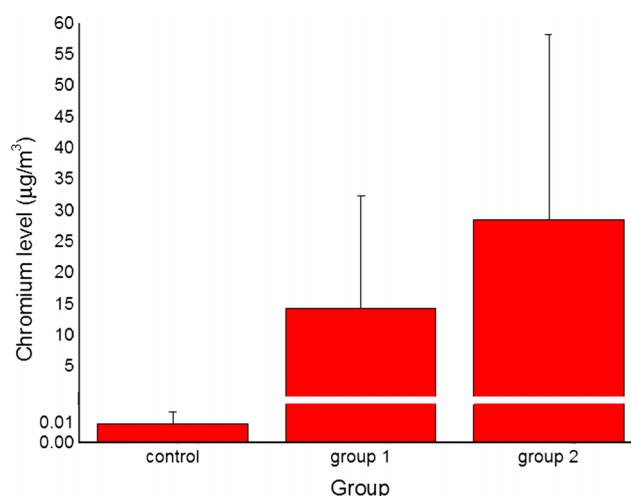


Figure 1 Chromium air concentrations for the exposed groups and the control group. The 50 workers in group 1 underwent cytokine analysis, while the 56 workers in group 2 underwent analysis for humoral immunity. The whiskers indicate chromium level variance. The chromium level in the general environment was close to zero, while chromium concentrations in group 1 ($14.38 \pm 18.43 \mu\text{g}/\text{m}^3$) and group 2 ($28.55 \pm 29.70 \mu\text{g}/\text{m}^3$) were higher than that in the control group ($p < 0.01$), but were still within the permissible range ($\leq 50 \mu\text{g}/\text{m}^3$).

Linear correlation

Correlation between the levels of cytokines, immunoglobulin and complement release and exposure biomarkers (WB-Cr, U-Cr) was analysed. As shown in figures 2 and 3, a negative correlation was found between log-WB-Cr and IL-17A, IFN- γ , IgG and IgA, ($r = -0.244$, $p = 0.016$; $r = -0.245$, $p = 0.045$; $r = -0.325$, $p = 0.002$; and $r = -0.231$, $p = 0.031$, respectively). A negative correlation was also found between log-U-Cr and IL-10 ($r = -0.250$, $p = 0.040$). Meanwhile, a positive correlation was shown between log-WB-Cr and serum levels of C3 and C4 ($r = 0.352$, $p = 0.001$ and $r = 0.276$, $p = 0.010$, respectively). Log-WB-Cr and log-U-Cr were used as they can be changed to a normal distribution after log transformation.

As cytokines, immunoglobulin and complement are associated with chromate carcinogenesis, and the micronucleus is an important biomarker for genetic damage, a correlation study was conducted between all cytokines in our study and the incidence of micronuclei. The results showed that there was a significant inverse relationship between the incidence of micronuclei and IL-10 and IL-17A ($r = -0.329$, $r = -0.312$, respectively).

Table 1 General characteristic of groups 1 and 2 and the control group

	Non-exposed (n=50)	Group 1 (n=50)	p Value	Group 2 (n=56)	p Value
Age, mean $\pm$ SD	39.4 $\pm$ 8.5	40.8 $\pm$ 5.2	0.494	39.4 $\pm$ 8.5	0.675
Years working, mean $\pm$ SD	–	17.4 $\pm$ 7.7		–	
Smoking status, n (%)					
Yes	29 (58.0%)	42 (85.7%)	0.003	29 (58.0%)	0.844
No	21 (42.0%)	7 (14.3%)		21 (42.0%)	
Alcohol intake, n (%)					
Yes	27 (54.0%)	43 (83.8%)	<0.001	27 (54.0%)	0.845
No	23 (46.0%)	6 (12.2%)		23 (46.0%)	
WB-Cr ($\mu\text{g}/\text{l}$), median (quartile)	1.74 (1.29)	14.8 (13.9)	<0.001	16.2 (15.1)	<0.001
U-Cr/creatinine ($\mu\text{g}/\text{g}$), median (quartile)	0.92 (0.51)	10.86 (8.79)	<0.001	16.28 (12.35)	<0.001

Table 2 Serum cytokine, immunoglobulin and complement in groups 1 and 2 and the control group

	Group 1 (n=50)		Group 2 (n=56)		Control (n=50)		p Value
	Mean±SD	Median (quartile)	Mean±SD	Median (quartile)	Mean±SD	Median (quartile)	
IL-2 (pg/ml)	1.24±0.11				1.25±0.18		0.811
IL-4 (pg/ml)	1.37±0.20				1.42±0.29		0.311
TNF- α (pg/ml)		1.60 (0.32)				1.50 (0.33)	0.880
IL-6 (pg/ml)		2.05 (0.73)				2.45 (1.15)	0.021
IL-10 (pg/ml)	1.68±0.38				1.82±0.34		0.045
IFN- γ (pg/ml)	3.06±0.73				3.46±0.91		0.032
IL-17A (pg/ml)	6.08±1.92				7.56±2.90		0.004
IFN- γ /IL-4	3.13±0.58				3.48±0.92		0.026
IgG (g/l)			10.94±2.45		12.41±2.05		0.026
IgA (g/l)			2.35±0.88		2.82±1.15		0.043
IgM (g/l)			1.17±0.49		0.97±0.43		0.246
IgE (g/l)				55.19 (157.41)		81.87 (237.08)	0.610
C3 (g/l)			1.20±0.24		0.91±0.13		0.001
C4 (g/l)			0.32±0.07		0.23±0.05		0.001

The cytokines had a reciprocal influence on each other: the levels IL-2, IL-4, IL-10, IFN- γ and IL-17A in the serum of all individuals correlated with each other ($p < 0.05$).

To determine immunoglobulin and complement alterations in relation to cytokine changes, 20 pairs of subjects with similar age, smoking status and other important exposure levels were selected from the group 1 and control group and a correlation study of cytokines (IL-6, IL-10, IFN- γ , IL-17A, IFN- γ /IL-4) and humoral immune factors (IgG, IgA, C3, C4) was carried out (see online supplementary table 4).

DISCUSSION

It is much easier for Cr-VI to enter cells than trivalent chromium (Cr-III). When Cr-VI is taken up by a lymphocyte, it is reduced to Cr-III and the reactive intermediate species produced could be harmful to the lymphocyte. At a molecular level, when Cr-VI enters the lymphocyte protein products such as cytokines, immunoglobulin and complement, may be affected.¹⁹ Tanigawa *et al* studied 19 male chromate workers and found that total T lymphocyte and total lymphocyte counts were decreased, and CD4 and CD8 T lymphocytes were also reduced in number.²⁰

Carroll *et al* found that both T and B lymphocytes were inhibited by K₂CrO₄ in vitro.²¹

Long-term exposure to chromium can increase the body's internal load. Irrespective of route of entry, chromium eventually enters the blood stream before being excreted in urine. In this study levels of WB-Cr and U-Cr, used as indicators to measure the chromate level in workers, were significantly higher in groups 1 and 2 than in the control group ($p < 0.001$).

Many researchers have found that chromium can cause a variety of harmful effects and even increase the incidence of human cancer.^{22–24} The development of cancer is a multi-stage and long term process, which is also related to many factors including the immune system.²⁵ The immune system helps maintain the stability of our internal environment and many diseases can result if this balance is disturbed.

Cytokines transmit different signals involving various signal pathways and have different biological effects.²⁶ IL-2, TNF- α and IFN- γ are all Th1 type cytokines, while IFN- γ , a pro-inflammatory cytokine, can help target cells generate various chemotactic factors to relieve the inflammatory response.^{27–28} TNF- α is a pleiotropic cytokine critical for

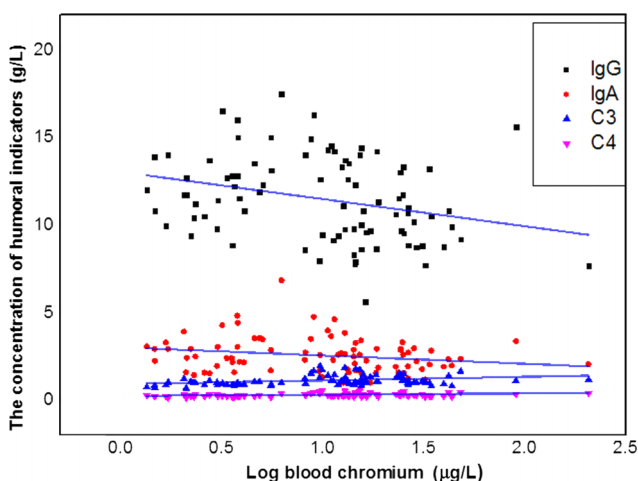


Figure 2 Correlation between cytokines (IL-17A, IFN-, IL-10) and exposure biomarkers (WB-Cr, U-Cr).

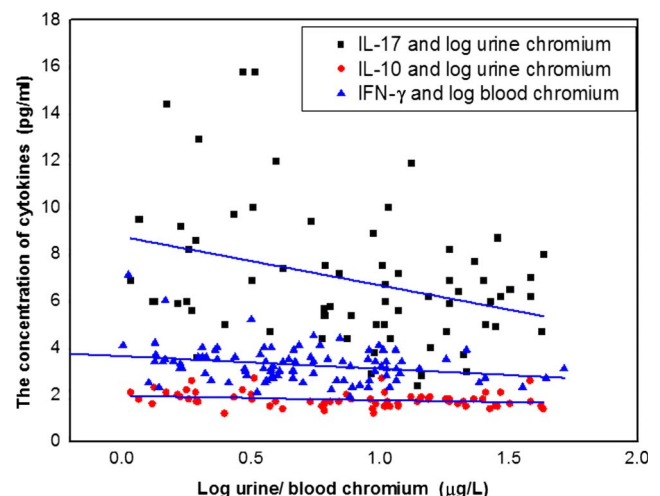


Figure 3 Correlation between humoral factors (IgG, IgA, C3, C4) and blood chromium concentration (WB-Cr).

inflammation, lymphoid neogenesis, and host defence against various pathogens, and plays multiple roles in the induction of cytokines IL-1, IL-6 and IL-8, and the activation of macrophages and neutrophils. These are all important for homeostasis of Th1 cells and antagonism of Th2 cells. Th1 cells also have an anti-tumour function, so when their number is reduced, the body is more susceptible to intracellular pathogens and is at risk of tumour genesis.²⁹

IL-4, IL-6 and IL-10 are cytokines secreted by Th2. They contribute to the induction of allergy and also help clear away extracellular parasites. IL-4 is anti-inflammatory and is a protection cytokine for immunoglobulin class switching. It can also promote the production of IgG and IgE, and inhibit the cell-mediated immune response and Th17 development. IL-6 is commonly involved in inflammatory and immune responses, and is produced by multiple cell types including T cells, B cells, monocytes and fibroblasts.^{30–34} IL-10, a cytokine with anti-inflammatory properties, plays a critical role in infection by limiting the immune response to pathogens and preventing undue damage to the host.³⁵ A decrease in IL-10 numbers can cause immune pathology due to uncontrolled inflammation.

IL-17A is a Th17 type cytokine. It is pro-inflammatory and has a protective role in lung immunity. It can also promote the mobilisation of neutrophils, cytokine production by epithelial cells, and angiogenesis. When numbers are reduced, the body is susceptible to extracellular pathogens.³⁶

In our study, all the above immune factors were analysed in a population of chromate production workers as well as a control group. The results showed that the Th1/Th2/Th17 cytokine profile had been changed (generally decreased), and a negative association with chromate exposure level was demonstrated, which can further affect the balance of cytokine regulation and the clearance of microbes.³⁷

Alteration or suppression of the immune system, especially through chronic changes, can induce dysfunction or result in the development of some diseases (eg, inflammatory conditions, cancer, etc). From the Th1/Th2/Th17 cytokine profile described above, it is known that IFN- γ , IL-6, IL-10 and IL-17A are all closely related to inflammation. The immunosuppression found in workers exposed to chromate was found to be associated with risk of inflammation as shown by the high incidence of damage to the nasal mucosa.¹⁷ Inflammation caused by chromate exposure is also shown in animal studies.³⁸ The authors demonstrated that Cr associated with stainless steel welding fumes was the main cause of the suppression of lung defence responses. Altered cytokine production has also been found during lung immune responses.

Some studies have found that Th1 and Th2 cell disturbance, especially the decreased in Th1 and increase in Th2 cells, further suggests that those affected are potentially at risk of cancer.^{39–40} The IFN- γ /IL-4 ratio reflects the Th1/Th2 ratio, because IFN- γ is predominately secreted by Th1 cells while IL-4 is the product of Th2 cells. When cytokine production is inhibited, the IFN- γ /IL-4 ratio is also reduced, reflecting Th1/Th2 disturbance. This is supported by the results of the correlation analysis between cytokines and micronucleus.

A micronucleus contains genetic material derived from the centromere and the entire chromosome.⁴¹ The generation of micronuclei in peripheral lymphocytes is an indication of cellular genetic toxicity. Our study found that IL-10 and IL-17A levels in chromate exposed workers were negatively correlated with micronuclei, which may indicate that the genetic damage caused by chromate exposure is related to immunotoxicity. Long term chromate exposure may cause respiratory system cancers, especially

lung cancer. The inhibition of cytokine production accompanying the increased incidence of micronuclei further underlines the risk of carcinogenesis in chromate exposed workers.

In humoral immunity, immunoglobulin can combine with antigens in an anti-infection response accompanied by complement activation. Increasing levels of C3 and C4 (two of the 11 human complements) are often apparent in the acute phase of inflammation. Immunoglobulin is the principal component of humoral immunity and a decrease in levels generally indicates a drop in humoral immunity. On the other hand, complement is responsible for inflammation progression. The results of this study show that both IgG and IgA were significantly decreased, IgM and IgE were both decreased but not significantly, and both C3 and C4 were increased in chromate exposed workers. Inverse associations also have been observed between IgG, IgA and WB-Cr, and positive associations between C3, C4 and WB-Cr. Humoral immunity was also altered or suppressed by chromate.^{42–43}

Many confounding factors may affect immune parameters such as cytokines and antibodies. Therefore we investigated whether smoking status or alcohol intake influenced the generation or expression of cytokines in our study. The stratified analysis showed that smoking status did not affect Th1/Th2/Th17 secretion, as the results before and after stratification were similar.

CONCLUSIONS

These findings suggest that the toxic effect of chromate on the immune system might result from the inhibition of cytokine secretion and immunoglobulin production, and the activation of complement generation, which are all altered by elevated chromate exposure. The effect of chromate on the immune system may be a direct result of the destruction of lymphocytes as shown by a high incidence of micronuclei, at least in peripheral lymphocytes.

The alteration of immune factors found in this study indicates that occupational chromate exposure may cause cellular and humoral immunity dysfunction, which could further indicate an elevated risk of inflammation and carcinogenesis. This issue should be further investigated in a cohort study.

Contributors All the authors fulfil the criteria for authorship. QQ and PL conceived and carried out the study, analysed the data and wrote the manuscript. TCW performed examinations. TC, LY, YSS, XHL, YGG and WY provided epidemiological support. GJ is responsible for the conduct of the study and the overall content. JZ and SFY helped with onsite investigation. All authors commented critically on the manuscript and agreed with the version submitted.

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Competing interests None.

Ethics approval The Biomedical Ethics Commission of the Faculty of Health Sciences (Peking University of Beijing, China) approved this study.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- 1 Gube M, Ebel J, Brand P, *et al.* Biological effect markers in exhaled breath condensate and biomonitoring in welders: impact of smoking and protection equipment. *Int Arch Occup Environ Health* 2010;83:803–11.
- 2 Andrea C, Matteo G, Olga A, *et al.* The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-plating workers. *Environ Health Perspect* 2006;114:542–6.
- 3 Shrivastava R, Upreti RK, Seth PK, *et al.* Effects of chromium on the immune system. *FEMS Immunol Med Microbiol* 2002;34:1–7.
- 4 Shashwat K, Awasthi SK, Srivastava JK. Effect of chromium on the level of IL-12 and IFN-gamma in occupationally exposed workers. *Sci Total Environ* 2009;407:1868–74.
- 5 Mohammad BH, Marie V, Gabriela C, *et al.* Low-Level environmental cadmium exposure is associated with DNA hypomethylation in Argentinean women. *Environ Health Perspect* 2012;120:879–84.

- 6 Liu XH, Song YS, Jia G, *et al.* Evaluation of the correlation between genetic damage and occupational chromate exposure through BNMN frequencies. *Genet Damage Occup Chromate Expo* 2012;54:166–70.
- 7 Marth E, Jelovcan S, Kleinhapfl B, *et al.* The effect of heavy metals on the immune system at low concentrations. *Int J Occup Med Environ Health* 2001;14:375–86.
- 8 Senovilla L, Vitale I, Martins I, *et al.* An immuno surveillance mechanism controls cancer cell ploidy. *Science* 2012;337:1678–84.
- 9 Mantovani A. Cancer: inflaming metastasis. *Nature* 2012;457:36–7.
- 10 Valentino M, Rapisarda V, Santarelli L, *et al.* Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers. *Human Exp Toxicol* 2007;26:551–6.
- 11 Yin Z, Zhang DH, Welte T, *et al.* Dominance of IL-12 over IL-4 in gamma delta T cell differentiation leads to default production of IFN-gamma: failure to down-regulate IL-12 receptor beta 2-chain expression. *J Immunol* 2000;164:3056–64.
- 12 Hueiwan AJ, Chih-Hong P, Norou D, *et al.* Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration. *Occup Environ Med* 2011;68:653–8.
- 13 Glaser U, Hochrainer D, Klöppel H, *et al.* Low level chromium (VI) inhalation effects on alveolar macrophages and immune functions in Wistar rats. *Arch Toxicol* 1985;57:250–6.
- 14 Katiyar S, Awasthi SK, Sahu RK. Suppression of IL-6 level in human peripheral blood mononuclear cells stimulated with PHA/LPS after occupational exposure to chromium. *Sci Total Environ* 2008;390:355–61.
- 15 Wu FY, Chang PW, Wu CC, *et al.* Correlations of blood lead with DNA-protein cross-links and sister chromatid exchanges in lead workers. *Cancer Epidemiol Biomarkers Prev* 2002;11:287–90.
- 16 Wang BJ, Sheu HM, Guo YL, *et al.* Hexavalent chromium induced ROS formation, Akt, NF- κ B, and MAPK activation, and TNF- α and IL-1 α production in keratinocytes. *Toxicol Lett* 2010;198:216–24.
- 17 Murgia N, Muzi G, Dellomo M, *et al.* Induced sputum, exhaled breath condensate and nasal lavage fluid in electroplating workers exposed to chromium. *Int J Immunopathol Pharmacol* 2006;19:67–71.
- 18 Mignini F, Tomassoni D, Traini E, *et al.* Immunological pattern alteration in shoe, hide, and leather industry workers exposed to hexavalent chromium. *Environ Toxicol* 2009;24:594–602.
- 19 Zhitkovich A. Chromium in drinking water: sources, metabolism, and cancer risks. *Chem Res Toxicol* 2011;24:1617–29.
- 20 Tanigawa T, Araki S, Araki T, *et al.* Decreases of CD4- and CD8-positive T lymphocytes in retired chromate workers. *Am J Ind Med* 1995;27:877–82.
- 21 Snyder CA, Valle CD. Immune function assays as indicators of chromate exposure. *Environ Health Perspect* 1991;92:83–6.
- 22 Haney JT, Erraguntla N, Sielken RL, *et al.* Development of a cancer-based chronic inhalation reference value for hexavalent chromium based on a non linear-threshold carcinogenic assessment. *Regulation Toxicol Pharmacol* 2012;64:466–80.
- 23 Clancy HA, Sun H, Passantino L, *et al.* Gene expression changes in human lung cells exposed to arsenic, chromium, nickel or vanadium indicate the first steps in cancer. *Metallomics* 2012;4:784–93.
- 24 Chhabra D, Oda K, Jagannath P, *et al.* Chronic heavy metal exposure and gall bladder cancer risk in India, a comparative study with Japan. *Asian Pac J Cancer Prev* 2012;13:187–90.
- 25 Beaver LM, Stemmy EJ, Schwartz AM, *et al.* Lung inflammation, injury, and proliferative response after repetitive particulate hexavalent chromium exposure. *Environ Health Perspect* 2009;117:1896–902.
- 26 Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* 2009;9:271–85.
- 27 Nyland JF, Fillion M, Barbosa F, *et al.* Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. *Environ Health Perspect* 2011;119:1733–8.
- 28 Cao XT. Perspective of immunology. *People's Med Publishing House* 2009.
- 29 Tang Y, Liao C, Xu X, *et al.* Th1/Th2 Cytokine profiles in G+/G- bacteremia in pediatric hematology/oncology patients. *Pediatr Blood Cancer* 2012;58:50–4.
- 30 Tato CM, Cua DJ. Snapshot: cytokines I. *Cell* 2008;132:324.
- 31 Tato CM, Cua DJ. Snapshot: cytokines II. *Cell* 2008;132:500.
- 32 Tato CM, Cua DJ. Snapshot: cytokines III. *Cell* 2008;132:900.
- 33 Tato CM, Cua DJ. Snapshot: cytokines IV. *Cell* 2008;132:1062.
- 34 Tato CM, Cua DJ. Alternative lifestyles of T Cells. *Cell* 2008;9:1323–5.
- 35 Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010;10:170–81.
- 36 Tato CM, Cua DJ. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 2007;8:1390–7.
- 37 Bene L, Falus A, Baffy N, *et al.* Cellular and molecular mechanisms in the two major forms of inflammatory bowel disease. *Pathol Oncol Res* 2011;17:463–72.
- 38 Antonini JM, Roberts JR. Chromium in stainless steel welding fume suppresses lung defense responses against bacterial infection in rats. *J Immunotoxicol* 2007;4:117–27.
- 39 Satyam A, Singh P, Badjatia N, *et al.* A disproportion of TH1/TH2 cytokines with predominance of TH2, in urothelial carcinoma of bladder. *Urol Oncol* 2011;29:58–65.
- 40 Barbara G, Cremon C, Carini G, *et al.* The immune system in irritable bowel syndrome. *J Neurogastroenterol Motil* 2011;17:349–59.
- 41 Seoane AI, Dulout FN. Genotoxic ability of cadmium, chromium and nickel salts studied by kinetochore staining in the cytokinesis-blocked micronucleus assay. *Mutat Res* 2001;490:99–106.
- 42 Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc* 2007;2:1084–94.
- 43 Mandy F, Perelmutter L. Laboratory measurement of total human serum IgE. *J Clin Immunoassay* 1983;6:140–6.
- 44 Burdon DR. Immunoglobulin G: functional sites. *Mol Immunol* 1985;22:161–206.

supplemental files

Table 3 Correlation analysis among cytokines(r)

	IL-2	IL-4	IL-10	IFN- γ	IL-17A	IL-17/IFN- γ
IL-4	0.415*	——				
IL-10	0.318*	0.288*	——			
IFN- γ	0.201*	0.279*	0.217*	——		
IL-17A	0.236*	0.365*	0.394*	0.200*	——	
IgG			0.42			
IgA					0.355	0.44
C4			-0.46*			

* $P < 0.05$; All are done by Pearson correlation.

Table4 Stratified analysis of serum cytokine levels in group1 and control group (pg/ml)

	Smoking status-Yes			Alcohol intake-Yes		
	Group 1(n=43)		<i>P</i>	Group 1(n=44)		<i>P</i>
	Means	Median		Means	Median	
IL-6	2.10	2.50	0.023	2.05	2.50	0.017
IL-10	1.67	1.87	0.039	1.68*	1.85	0.042
IFN- γ	3.06	3.56	0.027	3.07*	3.64	0.029
IL-17A	5.71	7.68	0.004	5.86*	8.23	0.002
IFN- γ / IL-4	2.21	2.58	0.023	2.23*	2.65	0.021

Note:Mann-Whitney U nonparametric test for IL-6, t test for the others