BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 346-352 (2006)

Immune Responses to Trichloroethylene and Skin Gene Expression Profiles in Sprague Dawley Rats[1]

XIAO-YAN CHEN[*], ZHI-XIONG ZHUANG[+,2], XIAO-HUI WANG[+], AND JIN-ZHOU ZHANG[+]

[*]*Shenzhen Futian People's Hospital, Shenzhen 518033, Guangdong, China;* [+]*Shenzhen Center for Disease Control and Prevention, Shenzhen 518020, Guangdong, China*

Objective To characterize the immune reaction in SD rats exposed to trichloroethylene (TCE) and to identify the gene expression profiles involved in skin after TCE exposure. **Methods** Fifteen percent of TCE was injected intradermally into the rat back (100 μL/120 g) at intervals of 7 days. Whole blood was collected 24 h after the fifth or seventh intradermic administration of TCE. The percentages of CD4+ and CD8+ of T lymphocytes were measured by a flow cytometer. The concentrations of IFN-gamma and IL-4 in the serum were semi-quantified by ELISA. Total RNAs of skin samples at 3 h or 24 h after the seventh dose of TCE in SD rats were extracted, and gene expression profiles of these tissues were analyszed by rat toxicology U34 array of Affymetrix. **Results** Obvious decline of CD4+ in T lymphocytes was observed in the TCE-administer group. No significant concentration differences in IFN-gamma and IL-4 were found between TCE-treated and control rats. Gadd45a and Mel were significantly up regulated in skin tissue 24 h after TCE exposure. The expression regulation of immune response factors was as active as proteins associated with lipid metabolism and synthesis process in these skin samples of SD rats exposed to TCE. **Conclusion** T-helper type 1 cells mediate immune response can not be elicited in TCE-treated SD rats, but certain immune disorder can be induced.

Key words: Trichloroethylene; SD rat; CD4+/CD8+; IFN-gamma; IL-4; Gene expression profiles

INTRODUCTION

Trichloroethylene (TCE) is a volatile organic liquid used mainly as a metal-degreasing solvent. TCE has become an environmental contaminant in most underground and surface waters[1]. Immune disorders induced by TCE exposure, which is regarded as a delayed-type hypersensitivity (DTH) response, have attracted increasing attention in China because of both healthy and financial losses brought about by this ailment[2]. Till now, there is no definite proof to disclose the basic rules of TCE-associated immune disorders. One major restriction on the etiopathogenesis exploration of human occupational diseases is that tissue samples from patients are difficult to obtain for further investigation, but biological reactions in skin might be the key factors involved in the etiology. As an important means, animal models play an important role in biological, medical, and toxicological experiments.

In a previewed study[3], we found that certain immune disorders can be induced in Sprague-Dawley (SD) rats by exposure to TCE. This study aimed to clarify the characteristics of this immune response.

A decisive step for the immune response is to stimulate different T-cell subpopulations. CD4+ or CD8+ T-cells can be further subdivided by a distinct cytokine production. T-helper type 1 (Th1) cells predominantly produce cytokines, such as interferon-gamma (IFN-gamma), interleukin-2 (IL-2), and IL-12, which stimulate a cellular immune response. In contrast, T-helper type 2 (Th2) cells predominantly produce IL-4 and IL-5. These cytokines boost IgE-mediated allergic reaction and inflammation[4]. The Th1/ Th2 distinction contributes to a better understanding of immune reactions in various diseases. In this study, the ratio of T-cell subpopulations and the contents of some cytokines representing Th1/Th2 balance was measured in blood of the SD rats after TCE exposure.

Large scale of differential gene expression profiles between healthy and sick tissues may provide clues to define the mechanisms involved in

[1]This work was supported by "973" Project (No. 2002CB512903) and Shenzhen Bureau of Science and Technology, China (No. 200204121; No. 200304156).

[2]Correspondence should be addressed to Prof. Zhi-Xiong ZHUANG.

Biographical note of the first author: Xiao-Yan CHEN, female, born in 1966, associate chief technician, Ph. D., majoring in toxicology and laboratory medicine.

0895-3988/2006
CN 11-2816/Q

toxicogenomics. High-throughput technologies can be used in the research of TCE-related toxicogenomics. We chose Affymetrix GeneChip to explore the gene expression profile of the SD rat skins exposed to TCE.

MATERIALS AND METHODS

Animal Husbandry

Healthy SD rats of either sex, weighing 110±10 g, were purchased from Experimental Animal Center of First Military Medical University (Grade Ⅲ, Certificate Number: 2002A040) and allowed to acclimatize for 1 week before experimentation. Temperature and relative humidity in the animal room were maintained at 23℃±2℃ and 66±6%, respectively. The animals had free access to boiled water and sterilized rat chow. Vitamins A and C were supplemented in water at intervals of several days.

Methods

SD rats were divided into TCE-exposed, 2,4-dinitrofluorobenzene (DNFB)-exposed, and olive oil-exposed groups (n=6 in each). Fifteen percent of TCE solubilized in olive oil was injected intradermally (100 μL/120 g, 100 μL per dot) into the rat back 24 hours after the hair was shaved in TCE group at intervals of one week. The control group was injected intradermally with olive oil and 0.5 percent of 100 μL DNFB intradermally. TCE and olive oil solutions were mixed with an equal volume of Freund's complete adjuvant in the first administration.

Analysis of CD4+/CD8+ subsets Blood was collected from the orbital vein into a sterile Na2-EDTA blood collection tube by quickly removing the rat eyeball from the socket 24 h after the fifth or seventh intradermic administration of TCE, olive oil or DNFB. Pipetted mouse-anti-rat CD4+ conjugated to 10 μL fluorescein isothiocyanate (FITC) and mouse-anti-rat CD8+ conjugated to 10 μL R. phycoerythrin (R-PE) (Serotec, UK) was added into the bottom of the tube. Ten μL isotype matched negative control mouse IgG1-FITC and 10 μL IgG1 R-PE (Serotec, UK) were added into the bottom of another tube. One hundered μL of anticoagulated whole blood was added into the bottom of each tube, vortexed gently and incubated for 30 minutes in the dark at room temperature. One thousand and two hundred μL 1×FACS^{TM} lysing solution (BD Inc., UK) was added into the tubes, vortexed gently and incubated for 10 minutes in the dark at room temperature. The mixture in the tube was centrifuged at 1000 rpm for 5 minutes at room temperature; the supernatant was discarded. The pellet was washed with 2 mL sheath fluid (BD Inc., UK). The cells were finally resuspended in 500 μL sheath fluid. The data were acquired on a FACSCalibur (BD Inc., UK) and analyzed with CELLQuest software. The instrument was calibrated using two-color beads. All the data were represented as peripheral mononuclear blood cells gated on T lymphocytes using FSC and SSC dot plots. Before samples were required, the threshold was adjusted by samples stained with isotype matched negative control mouse IgG1-FITC and IgG1-PE.

Semi-quantification of IFN-gamma and IL-4 Blood was collected seprarately from the orbital vein into a sterile pyrogen-free tube 24 h after the fifth or seventh intradermic administration of TCE, DNFB or olive oil, and stored at 2℃-8℃. The tubes were centrifuged at 1500 rpm for 20 min; the separated sera were removed and stored at -70℃ for test. The rat IFN-gamma and IL-4 standards (Jingmei Biotech Co., China) were reconstituted by a two-fold serial dilution. The serial dilutions of IFN-gamma and IL-4 standards were 2500, 1250, 625, 312.5, 156.25, 78.125, and 0 pg/mL and 500, 250, 125, 62.5, 31.25, 15.625, and 0 pg/mL, respectively. Standards or serum samples were added in duplicate with 100 μL per well, and incubated for 2 h at 37℃. After 5 washes with washing buffer, 100 μL diluted biotinylated-antibody was added to each well, incubated for 1 h at 37℃ and washed. One hundred μL of the diluted streptavidin-HRP conjugate was added to each well, incubated for 30 minutes (for IL-4 analysis) or 45 minutes (for IFN-gamma analysis) at 37℃ and washed. Then 100 μL of substrate solution was added into each well, incubated for 15-25 minutes at 37℃ in dark, and 100 μL of stop solution was added to each well. The optical density of each well was determined within 5 minutes, using a microplate reader at 450 nm.

Skin sample collection At the seventh dose, the rats of both TCE experimental group and olive oil control group were intradermally injected with 15% TCE or olive oil into different areas (at least 3 cm apart) 24 h and 3 h before sacrification. Skins of the injected area from male rats (one in each group) were cut into pieces (the size was less than 0.5 cm). After washing 3 times with sterilized PBS buffer, the skin samples were frozen in liquid nitrogen for use.

Microarray assay for gene expression profiles The total RNA was isolated from the frozen skin samples with TRIZOL reagent (Invitrogen, USA) and cleaned by QIAGEN's RNeasy (Germany). Only when the A260:A280 ratio was 1.8-2.0 with spectrophotometric analysis and the 28S:18S ratio of ribosomal band intensities was at least 2:1 by electrophoresis, could the isolated RNA be applied for microarray. Double-stranded cDNA from 10 μg

total RNA was synthesized and cleaned before biotin-labeled cRNA was synthesized by *in vitro* transcription using a BioArray high yield RNA transcript labeling kit. Ten μg of the fragmented cRNA probe was incubated with hybridization mixture at 99℃ for 5 minutes; then the hybridization cocktail was transferred to 45℃ for 5 minutes. The probe array was incubated at 45℃ for 10 minutes with rotating; the buffer solution was removed from the probe array cartridge and filled with appropriate volume of the clarified hybridization cocktail. The probe array was loaded around rotisserie axis and rotated at 60 rpm for 16 hours. The hybridized cocktail was removed from the probe array, washed and stained following the instructions of Genechip Fluidics Station (Affymetrix). The probe arrays were scanned normalized and analysed using MAS 5.0 software.

Descriptive statistical data were calculated using the SPSS10.0 software package for Windows. $P<0.05$ was considered statistically significant.

RESULTS

Changes of Peripheral T Lymphocyte Subsets CD4+/CD8+ in SD Rats After TCE Exposure

The percentages of CD4+ and CD8+ in peripheral T lymphocytes of six SD rats after 5 doses of olive oil treatment were 43.62/20.17%, 48.18/23.38%, 34.42/17.04%, 42.32/21.48%, 44.37/18.59%, and 52.58/19.19%. When exposed to olive oil for 7 times, the percentages of CD4+/CD8+ were 38.89/18.74%, 33.60/17.47%, 33.66/16.61%, 40.95/14.97%, 42.13/15.39%, and 42.56/27.28%. The percentages of CD4+ and CD8+ in peripheral T lymphocytes of six SD rats after 5 administer of TCE were 37.64/20.56%, 32.28/12.95%, 34.21/12.96%, 36.54/16.56%, 39.89/21.21%, and 39.32/21.48%. When TCE was administrated 7 times, the percentages of CD4+/CD8+ were 21.18/13.75%, 28.62/14.80%, 21.76/14.49%, 22.06/14.97%, 23.24/10.02%, and 31.82/20.50% (Table 1). Obvious decline of CD4+ in T lymphocytes was found in the TCE-treated group, compared with the olive-oil-treated group. The percentages of CD4+/CD8+ in peripheral T lymphocytes of six DNFB-treated SD rats after the 7th dose were 21.18/13.75%, 21.48/16.21%, 26.61/14.51%, 24.78/16.41%, 21.33/14.26%, and 22.47/12.75%; the average percentages of CD4+ and CD8+ were 22.98±2.23% and 14.65±1.42%, respectively (Table 1), similar to those found in TCE-treated rats.

Concentrations of IL-4 and IFN-gamma in Peripheral Rat Serum After TCE Exposure

In the peripheral serum of rats treated with 5 doses of olive-oil, the concentrations of IL-4 and IFN -gamma were 1, 2, 5, 10, 2, and 2 pg/mL，and 0 5, 20, 18, 0, and 5 pg/mL respectively. In rats treated with 7 doses of olive oil, the peripheral serum IL-4 and IFN-gamma were 47, 3.5, 0.5, 2, 0.5, and 2 pg/mL, and 2, 15, 0, 0, 0, and 2 pg/mL, respectively. After 5 doses of TCE treatment, the concentrations of IL-4 and IFN-gamma in the rat peripheral serum were 1.8, 2, 2.5, 3, 1, and 2 pg/mL，and 18, 18, 2, 2, 2, and 2 pg/mL, respectively. When rats were treated with 7 doses of TCE, the concentrations of IL-4 and IFN-gamma in their peripheral serum were 1.8, 3, 2, 2.5, 2, and 3 pg/mL, and 0, 18, 2, 2, 0, and 2 pg/mL, respectively. When rats were treated with 7 doses of DNFB, the concentrations of IL-4 and IFN-gamma in their peripheral serum were 0, 0.5, 2, 0.5, 2, and 1.8 pg/mL, and 2, 3, 2, 2, 3, and 2 pg/mL, respectively. No significant difference in cytokines was found by

TABLE 1

Changes of Peripheral T Lymphocyte Subsets CD4+, and CD8+ in SD Rats After 5th and 7th Exposures ($\bar{x} \pm s$)

Groups	Exposure Doses	Number of Rats	CD4+ (%)	CD8+ (%)
Olive-oil-treated	5	6	44.25±6.09	19.98±2.24
	7	6	38.63±4.08*	18.41±4.56$^{\#}$
TCE-treated	5	6	36.23±2.96**	17.62±4.03$^{\#\#}$
	7	6	24.78±4.39***	14.76±3.36$^{\#\#\#}$
DNFB-treated	7	6	22.98±2.23****	14.65±1.42$^{\#\#\#\#}$

Note. *No significant difference in peripheral CD4+ percentages between the rats treated with 7 and 5 does of olive-oil ($P>0.05$). **The peripheral CD4+ percentages in rats treated with 5 doses of TCE differ significantly from those treated with 5 doses of olive oil ($P<0.05$).***The peripheral CD4+ percentages in rats treated with 7 doses of TCE differ significantly from those treated with 7 doses of olive oil ($P<0.05$). ****The peripheral CD4+ percentages in rats treated with 7 doses of DNFB differ significantly from those treated with 7 doses of olive oil ($P<0.05$). $^{\#}$No significant difference in peripheral CD8+ percentages between rats treated with 7 and 5 doses olive-oil ($P>0.05$). $^{\#\#}$No significant difference in peripheral CD8+ percentages between the rats treated with 5 doses of TCE and olive-oil ($P>0.05$). $^{\#\#\#}$No significant difference in peripheral CD8+ percentages between the rats treated with 7 doses TCE and olive-oil ($P>0.05$). $^{\#\#\#\#}$No significant difference in peripheral CD8+ percentages between the rats treated with 7 doses of DNFB and olive-oil ($P>0.05$).

Student's *t* test. However, two rats in olive-oil-treated group had higher serum levels of IL-4 (10 and 47 pg/mL) than those in other rats. The serum levels of IL-4 in TCE- and DNFB-treated rats were lower than 3 pg/mL.

Gene expression profiles in skin tissues of SD rats after TEC exposure Significantly up- or down-expressed genes were selected according to the following criteria: (1) the absolute value of the Signal Log Ratio (SLR) equaled to or over 1, and the genes must be PRESENT. (2) the expression of the genes had remarkable changes from ABSENCE (A) to PRESENCE (P), or *vice versa*.

The number of up-expressed genes in skin tissues 3 h after TCE treatment (94 genes) was less than that 24 h after TCE-treatment (152 genes). A total of 169 genes were down-expressed in the skin samples 3 h after TCE treatment, and 109 genes in the skin samples 24 h after TCE treatment. In the skin tissues exposed to TCE for 3 h, immune response factors and receptors (IL-6 and insulin-like growth factor 2 receptor), protein/amino acid phosphorylase (Mapk12), and some electron tranport factors (*e.g.* cytochrome *c* oxidase, flavoprotein-ubiquinone oxidoreductase) (Table 2), were up-expressed. Some genes of phase I (some cytochrome enzymes) and phase II metabolic enzymes (*e.g.* glutathione-S-transferase), stress response factors, and some immune response receptors (*e.g.* beta-receptor of transforming growth factor) (Table 3), were down-expressed. Twenty-four hours after TCE treatment, genes of cytosolic epoxide hydrolase, enzymes related to metabolism or synthesis of lipid and steroid, glutathione transferase or reductase, apoptosis-related factors, stress response proteins, and Gadd45a (Table 4) were up-expressed. The down-expressed genes included mainly some P450 family members involved in lipid metabolism, some immune response factors and receptors, and Map2k5 (Table 5).

DISCUSSION

The key to control or promotion of allergic response is the polarized CD4+ T cells Th1 or Th2 lymphocytes. Delayed contact hypersensitivity is commonly regarded as an *in vivo* manifestation of type 1 cytokine-mediated immunity to epicutaneously applied haptens[5]. Studies with MRL +/+ autoimmune-prone mice[6] indicated that after 4 weeks of TCE treatment, the level of CD4+ T cells and secretion of IFN-gamma were high. In this study, the concentrations of IFN-gamma and IL-4 in SD rats treated with TCE had no significant difference from those in olive-oil and TCE -treated rats. The percentage of activated CD4+ T cells did not increase. On the contrary, obvious decline of CD4+ in T lymphocytes was found in TCE-treated rats, compared with the olive-oil-treated rats. There was no evidence that Th1-mediated response was elicited in TCE-treated SD rats. It could be concluded that the immune response in SD rats after TCE exposure differed from that in autoimmune-prone MRL +/+ mice. In some studies on acute or chronic phases of contact hypersensitivity[7], decline of CD4+ T cells in draining lymph node cells was observed when mice were treated with 2,4,6-trinitro-1-chlorobenzene. The results of this study are partly consistent with the results of Hideki Kitagaki *et al*.

Two rats in control group had higher concentrations of IL-4 (10 and 47 pg/mL) than those of other rats. The concentrations of IL-4 in TCE and DNFB-treated rats were lower than 3 pg/mL. These results reveal that TCE exposure inhibits the secretion of Th2-mediated IL-4 in SD rats. It was found that in arthritis rats treated with complete Freud's adjuvant, the expression level of IL-2 mRNA

TABLE 2

Significant Up-expressed Genes in SD Rats Exposed to TCE 3 h Before Sacrifice

Category or Functions	Gene Descriptions	SLR	Probe Set
Phase II Metabolism	*GST13-13* (Glutathione S-transferase, Mitochondrial)	2.4	S83436_i
Electron Transport	*Cox6a2* (Cytochrome *c* Oxidase)	1.2	rc_AI171644_s
	Cox 8 h (Cytochrom *c* Oxidase Subunit VIII-H (heart/muscle))	1.7	U40836mRNA_s
	Probable Flavoprotein-ubiquinone Oxidoreductase	1.5	rc_AI237007
Immune Response Factors and Receptors	*IL-6* (interleukin 6)	0.5	M26744
	Retinoid X Receptor Gamma	0.8	AJ223083
	Igf2r (Insulin-like Growth Factor 2 Receptor)	0.5	U59809_s
	IL-15 (Interleukin 15)	0.8	U69272_g
Protein Phosphorylase	*Mapk12* (Mitogen-activated Protein Kinase 12)	2.0	rc_AA849716

TABLE 3

Significantly Down-expressed Genes in SD Rats Exposed to TCE 3 h Before Sacrifice

Category or Functions	Gene Descriptions	SLR	Probe Set
Electron Transport	NAD(P) Quinone Reductase mRNA	1.8	M58495mRNA
Drug/xenobioctics	*Cyp3a3*	-1.0	D13912_s
Metabolism	*Cyp2c*	-0.8	M18363cds_s
Estrogen Metabolism	*Cyp1b1*	-1.0	X83867cds_s
Phase II Metabolism	*Gsta2* (Glutathione-S-transferase, Alpha Type2)	-1.1	S82820mRNA_s
	Nat1 (N-acetyltransferase 1)	-0.8	U01344
	LOC286989(UDP-glucuronosyltransferase)	-2.0	U27518
Amino Acid Synthesis	Phosphoserine Aminotransferase mRNA	-1.0	rc_AI102868
Glutathione Synthesis	*Gclc* (Glutamate-cysteine Iigase Catalytic Subunit)	-0.9	D14015_g
Transcription Factor	Signal Transducer and Activator of Transcription 6 (stat6) Gene	-1.1	AF055292mRNA
Immune Response	*Tgfbr2* (Transforming Growth Factor, Beta Receptor 2)	-1.1	L09653_s
Factors and Receptors	Madh1 (MAD Homolog 1) (*Drosophila*)	-1.4	AF067727_s
	IL-1rap (Interleukin 1 Receptor Accessory Protein)	-2.3	U48592_g
Stress Response	*Orp150* (Oxygen Regulated Protein 150kD)	-0.8	U41853
	Stress-70 Protein; Mitochondrial Precursor (75 kDa Glucose Regulated Protein)	-0.8	rc_AA944973
Apoptosis	Unknown mRNA Upregulated During Prostatic Apoptosis	-2.8	AJ224441UTR#1
Oncogene	*Bcl2* (B-cell Leukemia/Lymphoma 2)	-5.2	L14680

TABLE 4

Significantly Up-expressed Genes in SD Rats Exposed to TCE 24 h Before Sacrifice

Category or Functions	Gene Descriptions	SLR	Probe Set
Epoxide Metabolism	*Ephx2* (Cytosolic Epoxide Hydrolase)	3.2	X60328_g
	Cdo1 (Cytosolic Cysteine Dioxygenase)	1.1	E03229cds_s
Phase I Metabolism	Weak Similar to Ubiquinone Oxidoreductase MLRQ Subunit	1.4	AA686870_f
Lipid/Fatty Acid Metabolism Related	*Ehhadh* (Enoyl-Coenzyme A)	0.9	K03249
	Acadl (Acetyl-Coenzyme A Dehydrogenase)	1.1	J05029_s
	Cyp2b15 (Found Only in Keratinocytes)	1.2	X63545
	Me1 (Malate Metabolism)	1.06	rc_AI171506
Phase II Metabolism	*Gsr* (Glutathione Reductase)	0.6	U73174
	Gstm5 (Glutathione S-transferase, mu 5)	1.1	U86635
Transcription Factor	*Thra* (Thyroid Hormone Receptor Alpha)	0.6	M31174
	Similar to Activator 1 37 kDa Subunit	1.3	rc_AA819500_g
Apoptosis Activator	*Bok* (Bcl-2-related Ovarian Killer Protein)	2.0	AF027954
DNA Damage Related	*Dnase1l3* (Deoxyribonuclease I-like 3)	0.7	U75689_s
	Gadd45a (Growth Arrest and DNA-damage-inducible 45 Alpha, Sensory Injured Related)	0.9	rc_AI070295
Stress Response	*Hspt70* (Testis-specific Heat Shock Protein-related Gene hst70)	4.4	X15705cds
Endopeptidase Activity	*Psmb4* (Proteasome)	1.8	L17127

TABLE 5

Significantly Down-expressed Genes in SD Rats Exposed to TCE 24 h Before Sacrifice

Category or Functions	Gene Descriptions	SLR	Probe Set
Phase I Metabolism	*Akr1d1* (Aldo-keto Reductase Family 1)	-1.4	D17309
Cytochrome	*Cyp2f1*	-1.4	AF017393
Steroid/Lipid Associated Metabolism	*Cyp4b1*	-1.4	M29853
	Cyp4a12	-0.9	M37828
	Cyp2J3 (Cytochrome P450 Monooxygenase)	-0.5	U40004_s
	IL-6 (Interleukin 6)	-1.2	M26744
Immune Response	*IL-1b* (Interleukin-1 Beta)	-1.5	M98820
Factors and Receptors	*IL-8rb* (Interleukin 8 Receptor, Beta)	-0.7	U70988cds_s
	Tgfb1 (Transforming Growth Factor, Beta 1)	-2.5	X52498cds
Transcription Factor	*Rara* (Retinoic Acid Receptor, Alpha)	-1.0	U15211
Protein/Amino Acid Phosphorylation	*Map2k5* (Mitogen Activated Protein Kinase)	-0.9	U37462_s
Stress Response	*Orp150* (Oxygen Regulated Protein,150kD)	-0.8	U41853

is significantly elevated, but the expression levels of IFN-gamma, IL-10, and IL-4 are significantly decreased[8]. Imbalance of cell-mediated immunity, especially the Th1/Th2 and Th/Ts is regarded as one of the main pathological characteristics of arthritis rats.

With the introduction of high-throughput technologies for analysis of gene expression, PCR-based suppression subtractive hybridization and microarray techniques have been applied to toxicological investigation of TCE exposure. Chips containing 148 genes were used to examine gene expression in the liver of male Swiss Webster mice[9]. However, only three genes (Cyp2a, Hsp 25, and Hsp 86) have been induced by the highest dose. With the aid of Affymetrix RT-U34 microarrays, more regulaled expression genes can be disclosed in rats. Mel is the gene of malic dehydrogenase, a regularly controlled enzyme in tricarboxylic acid cycle. The level of malic dehydrogenase decreases in rat heart when the rats receive carnosine[10]. In this study, Mel gene was significantly up-expressed in SD rats exposed to TCE. As a regularly controlled enzyme in tricarboxylic acid cycle, an important biological cycle associated with fat metabolism, Mel gene is reasonably considered as a biomarker of TCE exposure in SD rats.

The gene expression regulation of immune response factor was active in SD rats exposed to TCE; 3 h after TCE treatment, immune response factors (IL-6, IL-15, and insulin-like growth factor 2 receptor) were up-expressed (Table 2). Twenty four h after TCE treatment, no immune response factor-related genes were up-expressed, but the expressions of IL-6, IL-1b, and transforming growth factor beta 1 were significantly lower (Table 5). Cytokines are important mediators involved in the regulation of the acute-phase response to injury and infection[11]. These cytokines also play a crucial role in haematopoiesis, hepatic and neuronal regeneration, embryonal development and fertility. Dysregulation of cytokine signalling leads to the onset and maintenance of several diseases, such as rheumatoid arthritis, inflammatory bowel disease, osteoporosis, multiple sclerosis, and cancer. *IL-6* type cytokines exert their action *via* the signal transducers glycoprotein 130, mitogenactivated protein kinase (MAPK) cascades, and other receptors. The result of microarray analysis in this study might imply certain immunological response pattern correlated with TCE exposure in SD rats.

Most significantly expressed genes are associated with enzyme-related metabolism or synthesis of lipid and steroid both in liver, suggesting that lipid metabolism is triggered when SD rats are exposed to TCE. It was reported that acute exposure to TCE induces lipid peroxidation in the liver of SD rats[12]. Low dose TCE exposure leads to lipid peroxidation in human beings[13]. After long (12 and 24 weeks) TCE inhalation exposure in rats, necrotic lesions with fat changes have been observed[14].

REFERENCES

1. Peters R J (2003). Chloroacetic acids in European soils and vegetation. *J Environ Monit* **5**(2), 275-280.
2. Li Laiyu, Lin Bingjie, Huang Xianqing, *et al*. (2002). Prevention measures of occupational mentosa-like dermatitis cause by trichloroethylene exposure. *Chin Occup Med* **29**(3), 59-60. (In Chinese)
3. Chen Xiao-yan, Zhuang Zhi-xiong, Huang Hai-xiong, *et al*. (2004). Model of immuno-response elicited by trichloroethylene in SD rat. *Chin Occup Med* **31**(2), 29-31. (In Chinese)
4. Liu Jinbao, Zhong Nanshan, Li Shunong (2000). The specific outer indicator and the identification of Th1/Th2 subset cells.

Chinese J of Pathophysiology **16**(8), 757-760. (In Chinese)
5. Moodycliffe AM/SE Ullrich (1995). Role of cytokines in the regulation of hypersensitivity responses.In Human Cytokines: Their Role in Disease and Therapy. B A Aggarwal and R J Puri, eds, Blackwell, Boston, pp. 131-152.
6. Gilbert KM, Griffin JM, Pumford NR (1999). Trichlorothylene activates CD4+ T cells: potential role in an autoimmune response. *Drug Metab Rev* **31**(4), 901-916.
7. Hideki Kitagaki, Momoko Kimishima, Yuichi Teraki, *et al*. (1999). Distinct in vivo and in vitro cytokine profiles of draining lymph node cells in acute and chronic phases of contact hypersensitivity: importance of a type 2 cytokine-rich cutaneous milieu for the development of an early-type response in the chronic phase. *J of Immunology* **163**, 1265-1273.
8. Fang Jian, Zhang Yong-Xiang, Ru Xiang-Bin, *et al*. (2000). Study of immune functions of adjuvant arthritis in rats. *Chinese J of Immunology* **10**(16), 525-528. (In Chinese)
9. Bartosiewicz M, Penn S, Buckpitt A (2001). Applications of gene arrays in environmental toxicology: fingerprints of gene regulation associated with cadmium chloride, benzo(a)pyrene, and trichloroethylene. *Environ Health Perspect* **109**(1), 71-74.
10. Lee J W, Miyawaki H, Bobst E V, *et al*. (1999). Improved functional recovery of ischemic rat hearts due to singlet oxygen scavengers histidine and carnosine. *J Mol Cell Cardiol* **31**(1), 113-121.
11. Peter C HEINRICH, Iris BEHRMANN, Serge HAAN, *et al.* (2003). Principles of interleukin (IL)-6-type cytokine signaling and its regulation. *Biochem J* **374**, 1-20.
12. Zhang Jinzhou, Huang Haixiong, Zhuang Zhixiong, *et al*. (2000). The effect of rat liver lipid peroxidation when acute exposed to trichloroethylen. *Chinese J of Public Health* **16**(4), 308-309. (In Chinese)
13. Huang Jinsheng, Chen Huizhen, Wu Pingfang, *et al*.(2002). The effect of low concentration trichloroethylene to human anti-peroxidase system. *Chinese J of Public Health* **18** (3), 282-283. (In Chinese)
14. Kumar P, Prasad AK, Mani U, *et al*. (2001). Trichloroethylene induced testicular toxicity in rats exposed by inhalation. *Hum Exp Toxicol* **20**(11), 585-589.

(*Received October* 20, 2005 *Accepted August* 23, 2006)