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Trichloroethylene-induced hypersensitivity dermatitis was associated with hepatic metabolic enzyme genes and immune-related genes

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Abbreviations:

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTLA-4, cytolytic T lymphocyte-associated antigen-4; Foxp3, forkhead box transcription factor; T-Bil, total bilirubin; TCA, trichloroacetic acid; TCE, trichloroethylene; SD, standard deviation; Treg, regulatory T cell; U-TCA, urinary trichloroacetic acid.

Abstract

Trichloroethylene (TCE) is one of the common organic solvents that has been widely used in cleaning or degreasing of metal products and electronic products. However, hundred cases of hypersensitivity dermatitis have occurred after the workers were occupationally exposed to TCE in China for the past decade. The purpose of this study was to investigate mRNA expression of hepatic metabolic enzyme genes, immune-related genes, apoptosis genes and oncogenes in patients with hypersensitivity dermatitis induced by trichloroethylene. 12 typical patients with TCE-induced hypersensitivity dermatitis were investigated as the study cases, peripheral blood samples were taken from patients and control, real-time fluorescent PCR assay was applied for detection of mRNA expression of hepatic metabolic enzyme genes, immune-related genes, apoptosis genes and oncogenes. It was found that the relative levels of mRNA expression of \textit{CYP1A2}, \textit{CYP2E1}, \textit{CYP3A4}, \textit{CYP2C9}
increased by 723%, 318%, 385%, 216%, respectively, when compared with control ($p<0.01$ or $p<0.05$); Foxp3, GATA3 and CTLA4 mRNA expression increased by 104%, 106%, 253%, respectively, in TCE patients when compared with control ($p<0.01$); T-bet expression decreased by 44% when compared with control ($p<0.01$); These findings indicate that some immune-related genes and hepatic metabolic enzyme genes might play an important role in the process of trichloroethylene-induced hypersensitivity dermatitis.

**Key words:** Trichloroethylene; hypersensitivity dermatitis; liver dysfunction; hepatic metabolic enzyme; immune-related genes; oncogene.
Introduction

Trichloroethylene (TCE) is a chlorinated organic solvent that has been widely used in industrial applications including cleaning and degreasing metal products and in computer-chip manufacturing. TCE is highly lipophilic and readily absorbed systemically through oral, dermal, or inhalation exposure. Because of its widespread commercial use and improper disposal, TCE has also become a major environmental pollutant. It has been reported that TCE could induce skin damage, liver damage, even heart or kidney impairment. Several epidemiologic studies have found the positive association between TCE exposure and the development of hypersensitivity dermatitis. In recent years, we have observed that some workers occupationally exposed to elevated concentrations of TCE might also be suffered from hypersensitivity dermatitis. These conditions were referenced as symptoms of chemically induced hypersensitivities. Most patients with dermatitis or liver impairment also have elevated enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The data suggest cases of TCE-induced hypersensitivity dermatitis and subsequent death have been increasing since the mid-1990s in China.

Although TCE toxicity is well studied, the mechanism of TCE-induced hypersensitivity dermatitis remains unclear. Our previous work found that only a few TCE-exposed people suffered from hypersensitivity dermatitis in relation to a liver impairment. This combination suggested that individual susceptibility or some aspect of TCE metabolism difference might be the critical factors closely related to disease occurrence. It also has been reported that TCE is metabolized mainly in the liver by cytochrome P450 mediated enzymes. In order to reveal the role of TCE in changing liver anabolic function and immune response, this study was undertaken to investigate the possible association of TCE-induced allergic disorder with hepatic metabolism gene, immune-related gene expression as well as other gene changes in those patients with TCE-induced disorder. Subsequently, we examined the alteration of mRNA expression of hepatic metabolism genes, immune-relatatory genes, apoptotic regulatory genes and oncogenes as well as mRNA expression in peripheral blood cells of TCE-exposed patients with allergic disorder. These were examined to reveal the
role of TCE in changing liver anabolic function and immune response.

Material and methods

Subjects and blood sample collection

This study was conducted with local ethics authority approval and patient consent. Twelve typical patients with TCE-induced hypersensitivity dermatitis were studied in Shenzhen city in south China. Among them, 5 were male and 7 were female with the average age of 24.3 years old. Seven patients were from the electronic industry, 3 from a hardware products factory, and 2 from other occupations. Additionally, 12 healthy workers (5 male and 7 female) without unknown or documented history of TCE exposure were chosen as the control group, their ages ranged from 20 to 26, the average age was 25.1 years old. Other conditions such as lifestyle, work time, etc. were similar to the TCE patients.

The most usual route of exposure was direct contact with TCE via skin or inhalation. In the electronic and metal products industries, TCE is extensively used as a cleaning and degreasing agent and the workers usually wear no personal protect equipment (i.e. rubber gloves or masks). Thus, respiration and skin contact is the main way to TCE exposure for the workers. The duration of TCE exposure among these patients varied individually. The period of TCE exposure ranged from 15 to 83 days (average 35.3 days).

Usually when any case of TCE-induced hypersensitivity dermatitis occurred, the TCE exposure concentrations in the workplace were investigated. We sampled and tested some cleaning agents from companies where TCE-induced skin disorder occurred. TCE contents in 3 samples were 37.5%, 91.4% and 63.5%, respectively. Additionally, air TCE levels in the workplace were also tested. The TCE air concentrations varied between 18 mg/m³ and 683 mg/m³ with the greater average value than the Chinese National Health Standard. About 6 patients were exposed to lower levels of TCE (10 to 25 mg/m³), while the other patients were exposed to levels of TCE that exceeded the limit of a time-weighted average (TWA) of 30 mg/m³ as recommended by the People's Republic of China (PRC) Ministry of Health (Occupational exposure limit for hazardous agents in the workplace, GBZ2.1-2007, China).
The symptoms of TCE-induced hypersensitivity dermatitis usually appear after 2 to 5 weeks exposure. Those symptoms include headache, dizziness, fever, skin itch of arms or legs or whole body, fatigue, anorexia, nausea and vomiting. Additionally, typical symptoms of skin damage include scarlet skin, erythema and rash. These changes became worse in 3~4 days. In some patients, their face and eyelids were swollen while the lips and mucous membrane of the mouth became so sore and inflamed that they could not open their mouths nor ingest food easily. Other symptoms included skin blisters which subsequently broke, exuded, scabbed, and finally became necrotized (See Table 2 and Table 3).

Approximately 5 ml of peripheral blood from each TCE patient and the control group were collected in heparin-coated tubes. The samples were stored at -80°C pending further analysis.

Reagents and instruments

Trizol reagent for total RNA extract was purchased from Invitrogen (Grand Island, USA), SYBR Green PCR kits were purchased from Takara (Dalian, China), primers of target genes and β-actin were synthesized by Shanghai Biological Engineering Co., Ltd (Shanghai, China) (Table 1). A real-time fluorescent quantitative PCR amplifier was bought from Applied Biosystem (ABI 7900HT, USA).

Table 1. Real-time PCR primer sequences and length of target genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
</table>
| β-actin | F: 5’-TAAAAGACCTCTATGCCAACACACAG -3’  
R: 5’-CACGATGGAGGGGCGGACACTATC -3’ | 122 |
| Foxp3 | F: 5’-CTCCTACCCACTGCTGGACAGCAAT-3’  
R: 5’-CCCTGCCCTTTCATCCAGA-3’ | 122 |
| GATA3 | F: 5’-CGAGATGGACGGGACACTA-3’  
R: 5’-TGGTCTGACAGTTGCACAGG-3’ | 142 |
| CTLA4 | F: 5’-GGATTTCAGCGCGCACAGG-3’  
R: 5’-CCTGGAGATGCATACTCACAACA-3’ | 179 |
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>PCR Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-bet</td>
<td>5'-TGTTGTGGTCAAGTTTAATCAGCA-3'</td>
<td>5'-CCCGGGCAGTAAATGACAG-3'</td>
<td>95</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>5'- ATCCTGGAGACC TTCCGACAC-3',</td>
<td>5'- TGACCTGCACCTTGTTACG-3'</td>
<td>130</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>5'-CTCTGAGATGGGCTCTGGATT-3'</td>
<td>5'-AATGGGTGTCGAGGTTCTC-3'</td>
<td>212</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>5'-GCACATAGCCAGCAAGAGA-3'</td>
<td>5'-CCAGGAGAAGGCCAGTTTCC-3'</td>
<td>121</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>5'-GGGAGGAAGCTGATGTTGAG-3'</td>
<td>5'-GGCAGAAGGCGAGGGTG-3'</td>
<td>171</td>
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<tr>
<td>CYP2C9</td>
<td>5'-AGGAGCATGAGGACCCCTTGA-3'</td>
<td>5'-GCACAGCCC AGGA TGAAGTG-3'</td>
<td>104</td>
</tr>
<tr>
<td>BAX</td>
<td>5'-GCAGAAGTCTCAGAGGCATC-3'</td>
<td>5'-CCAGTTGAAGTTGCGTCCAGA-3'</td>
<td>143</td>
</tr>
<tr>
<td>BAD</td>
<td>5'-TGAACCCGACATCTGACAC-3'</td>
<td>5'-TAGTGCACCGGCTTGAGA-3'</td>
<td>150</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>5'-TGAACCCGACATCTGACAC-3'</td>
<td>5'-CGTCTTCAGACAGCCCAAGG-3'</td>
<td>104</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>5'-GACTCTGGGAATATCCTGGACACA-3'</td>
<td>5'-CTGAGGCTGGCTGACATCA-3'</td>
<td>143</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>5'-ATGAGAAGGCGCTGTAGTAA-3'</td>
<td>5'-GACGATTTCCTCCCTGCTCT-3'</td>
<td>104</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>5'-ACACTTCTTCCCCTCAGGAG-3'</td>
<td>5'-CCCCATATGATCGACATCCA-3'</td>
<td>186</td>
</tr>
<tr>
<td>C-fos</td>
<td>5'-TCTTACTACCACTCACCCGCAGAC-3'</td>
<td>5'-GGATGAAAGTGGCCTGAGGAG-3'</td>
<td>104</td>
</tr>
<tr>
<td>C-Myc</td>
<td>5'-TCCACTACACCCATCGAGAC-3'</td>
<td>5'-GCGGAGGAAGTGGCCTGTGA-3'</td>
<td>145</td>
</tr>
<tr>
<td>K-ras</td>
<td>5'-GACCTGCTGTGTCGAAGATCACA-3'</td>
<td>5'-GAAGCTGAAGGGCGCTTGAGA-3'</td>
<td>144</td>
</tr>
<tr>
<td>p53</td>
<td>5'-GAGTGCCCTTCTGCTTGAGAC-3'</td>
<td>5'-GAGTGCCCTTCTGCTTGAGAC-3'</td>
<td>150</td>
</tr>
</tbody>
</table>

**Real-time quantitative PCR**
Total RNA from blood samples was extracted with Trizol according to the manufacturer’s specifications. Approximately 2 μg of RNA was used to synthesize the first-strand cDNA according to the protocol of reverse transcription kit. SYBR Green PCR kit was used for real-time quantification of target genes. The PCR reaction was conducted in a final volume of 25 μl, containing 0.5μl forward primer (10 mmol/L) and 0.5 μl reverse primer (10 mmol/L). A pair of conventional PCR primers for target genes was used for the amplification reaction on the real-time PCR amplifier. Real-time fluorescent PCR was performed with primers in Table 1 and cDNA as template. PCR reaction preparation was done as follows: 2×SYBR Green Q-PCR master mix 12.5 μl, forward primer (10 μmol/L) 0.5 μl, reverse primer (10μmol/L) 0.5 μl, ROX Reference Dye (50 ×) 0.5 μl, cDNA 2μl, ddH₂O 9 μl. The group with cDNA replaced by ddH₂O was used as no-template control. The following thermal cycling profile was used: denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and anneal for 30 s. The anneal and extension temperatures for each gene as follows: CYP1A2 54°C, CYP3A4 54°C, CYP2E1 55°C, BAX 54°C, BAD 57°C, Bcl-2 57°C, Caspase-3 54°C, Caspase-8 54°C, Caspase-9 54°C, c-fos 54°C, c-myc 54°C, K-ras 54°C, p53 54°C. The relative expression levels of the target gene mRNA were determined with reference to the expression of the β-actin gene.

Statistical Analyses

Statistical analysis was performed by comparing the TCE patients with the healthy control using SPSS 13.0. All data were presented as mean ± standard deviation (SD). Group differences were analyzed using one-way analysis of variance (ANOVA) followed by LSD’s post hoc tests. Differences between groups were considered significant when the p value<0.05.

Results

Clinical symptoms and signs of patients with TCE-induced allergic disorder

The positive number of patients with headache, dizziness, fatigue, nausea, vomiting, as well as appetite decrease and skin itch was 10, 12, 9, 7, 3, 10, and 12, respectively. Seven patients had elevated temperature with the highest temperature of 39.1°C, and the average temperature of 37.6°C among all patients. The skin lesion was the most typical sign. Among the 12 patients, 11 (91.7%) had scarlet skin, 10
(83.3%) had rash, 8 (66.7%) had erythema while 4 (33.3%) had blisters. Additionally, jaundice occurred in 3 patients (25.0%), liver enlargement occurred in 2 patients (16.7%) (Table 2, Table 3, Figure 1, Figure 2).

Table 2. The major symptoms and signs in the patients with TCE-induced hypersensitivity dermatitis (n=12)

<table>
<thead>
<tr>
<th>symptoms and signs</th>
<th>headache</th>
<th>dizziness</th>
<th>fatigue</th>
<th>nausea</th>
<th>vomiting</th>
<th>appetite decrease</th>
<th>skin itch</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive patients</td>
<td>10</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>positive rate (%)</td>
<td>83.3</td>
<td>100</td>
<td>75.0</td>
<td>58.3</td>
<td>25.0</td>
<td>83.3</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>symptoms and signs</th>
<th>fever</th>
<th>scarlet skin rash</th>
<th>erythema</th>
<th>blisters</th>
<th>jaundice</th>
<th>liver enlargement</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive patients</td>
<td>7</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>positive rate (%)</td>
<td>58.3</td>
<td>91.7</td>
<td>83.3</td>
<td>66.7</td>
<td>33.3</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Table 3. TCE exposure time and laboratory data in the patients with TCE-induced hypersensitivity dermatitis (n=12)

<table>
<thead>
<tr>
<th>TCE exposure (d)</th>
<th>Temp (°C)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>T-Bil (mg/L)</th>
<th>U-TCA (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>15~83</td>
<td>36.5~39.3</td>
<td>27~1865</td>
<td>21~785</td>
<td>12.5~63.7</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>35.3</td>
<td>37.6</td>
<td>382.3</td>
<td>115.3</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>SD</td>
<td>16.2</td>
<td>0.8</td>
<td>213.6</td>
<td>102.4</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Figure 1. Severe skin lesion on the limbs of the patient with trichloroethylene-induced hypersensitivity dermatitis.

Figure 2. Cutaneous manifestations on upper trunk of the patient with trichloroethylene-induced hypersensitivity dermatitis. Erythroderma, maculopapular eruption, exfoliative dermatitis and desquamation developed.

The abnormal rates of ALT, AST and T-bilirubin were 91.7%, 83.3% and 66.7%, respectively. Trichloroacetic acid (TCA), the most common metabolite of TCE was elevated in 8 of 12 patients with the highest concentration of about 86.2 mg/L. This value is greater than the normal value of urinary TCA (50 mg/L TCA in exposed workers where working conditions do not exceed the guideline recommended by China Ministry of Health, Table 3).

**Changes of hepatic metabolic enzyme mRNA expression**

Total RNA was extracted from peripheral blood of TCE patients, real-time fluorescent PCR was applied for determination of hepatic metabolic enzyme gene expression including *CYP1A2, CYP2E1, CYP3A4, CYP2C9* and *CYP2A6*. The results showed that the levels of *CYP1A2, CYP2E1, CYP3A4, CYP2C9, CYP2A6* were 8.23±2.49, 4.18±1.19, 4.85±1.09, 3.16±1.77, 1.63±1.24 fold of healthy control, respectively. The data indicated that *CYP1A2, CYP2E1, CYP3A4* and *CYP2C9* were
increased by 723%, 318%, 385% and 216%, respectively when compared with control ($p<0.01$ or $p<0.05$), but no significant difference was found for CYP2A6 mRNA expression between TCE patients and control, the details shown in Figure 3.

**Changes of immune-related gene mRNA expression**

mRNA expression of immune-related genes including Foxp3, GATA3, CTLA3 and T-bet was detected with real-time fluorescent PCR for TCE patients. The results showed that the levels of Foxp3, GATA3, CTLA3 and T-bet were 2.15±0.53, 1.97±0.61, 3.41±1.26 and 0.53±0.37 fold of that in control, respectively. When compared to the control, Foxp3, GATA3 and CTLA3 were increased by 115%, 97% and 241%, while T-bet was decreased by 47% in comparison with control ($p<0.01$) (Figure 4).

**Changes of apoptosis gene mRNA expression**

mRNA expression of apoptosis control genes including BAX, BAD, Bcl-2, Caspase-3, Caspase-8 and Caspase-9 were detected with real-time fluorescent PCR in TCE patients. The results showed that the levels of BAX, BAD, Bcl-2, Caspase-3, Caspase-8 and Caspase-9 were 1.85±0.42, 2.39±0.54, 0.73±0.21, 2.30±0.62, 3.31±0.69, 3.49±0.83 fold of control, respectively. The data indicated that BAX, BAD, Caspase-3, Caspase-8 and Caspase-9 were increased by 85%, 139%, 130%, 131% and 249%, respectively, when compared with control ($p<0.01$), while Bcl-2 was decreased by 27% in comparison with control ($p<0.05$) (Figure 5).

**Changes of oncogene mRNA expression**

Determination of mRNA expression of oncogenes including c-fos, c-myc, k-ras and p53 were conducted with real-time fluorescent PCR for TCE patients. The results showed that the levels of c-fos, c-myc, k-ras and p53 were 4.52±1.76, 1.41±0.22, 2.36±0.37, 1.64±0.26 fold of that in control, respectively. The data indicated that c-fos, c-myc and k-ras were increased by 352%, 41%, 136%, 64%, respectively, when compared with control ($p<0.01$ or $p<0.05$) (Figure 6).
Figure 3. mRNA expression of hepatic metabolic enzyme genes in peripheral blood from TCE-induced dermatitis patients and healthy controls. Total RNA was extracted from each patient, and real-time fluorescent PCR was applied for the gene amplification. The data were expressed as means ± SD. *p<0.05, **p<0.01 when compared with control.

Figure 4. mRNA expression of immune-related genes in peripheral blood from TCE-induced dermatitis patients and healthy controls. Total RNA was extracted from each patient, and real-time fluorescent PCR was applied for the gene amplification. The data were expressed as means ± SD. **p<0.01 when compared with control.
Figure 5. mRNA expression of apoptosis genes in peripheral blood from TCE-induced dermatitis patients and healthy controls. Total RNA was extracted from each patient, and real-time fluorescent PCR was applied for the gene amplification. The data were expressed as means ± SD. *p<0.05, **p<0.01 when compared with control.
Figure 6. mRNA expression of oncogenes in peripheral blood from TCE-induced dermatitis patients and healthy controls. Total RNA was extracted from each patient, and real-time fluorescent PCR was applied for the gene amplification. The data were expressed as means ± SD. *p<0.05, **p<0.01 when compared with control.

Discussion

Trichloroethylene has been used in industries for many years, and the toxicity of TCE has been extensively evaluated. Many studies have shown that TCE had harmful effects on liver, kidney, heart and skin. In recent years, studies have found that TCE was a potential human carcinogen and the International Agency for Research on Cancer (IARC) listed TCE as a carcinogen in group 2A. Skin damage is the most typical clinical feature of TCE-induced hypersensitivity dermatitis, which can be classified into four categories: exfoliative dermatitis, erythema multiforme, Stevens-Johnson syndrome, and epidermolysis bullos or toxic epidermal necrolysis. Actually TCE-induced hypersensitivity dermatitis belongs to delayed-type hypersensitivity (DTH) as described by Coombs and Gell. To some extent, TCE-induced hypersensitivity dermatitis is similar to drug- hypersensitivity dermatitis. Cutaneous manifestations often emerge as a morbilliform eruption. Simultaneously, the face, upper trunk, and upper extremities are the first locations to be affected, followed by the lower extremities. Erythroderma may occur, and the maculopapular eruption later becomes infiltrated and indurated while another clinical presentation is mucosal involvement.
Generally the symptoms in these patients appeared similar and varied in severity. Patients usually have headache, dizziness, fatigue, fever, and skin itch. Because these symptoms lack specificity, it is hard to make precise diagnosis at the early stage. However, skin damages including scarlet skin, erythema, rash and even skin blisters are the dominant signs as indicated by the present study. This kind of skin damage usually appeared 3~4 days after the dermatitis, which starts from the face and then extends the neck, arms, legs and trunk. It should be noted that TCE-induced skin damage is different from the contact dermatitis which emerged in and around the local contact area where direct chemical exposure occurred. Therefore, we must assume that the skin damage is a valuable and predictive factor for the diagnosis of TCE-induced hypersensitivity dermatitis.

On the other hand, trichloroacetic acid (TCA) is one of the principal TCE metabolites and represents a specific index for TCE exposure or absorption. We and other researchers found that urinary TCA levels did not reflect the severity of TCE-induced disorder, but TCA determination may be helpful for making a correct diagnosis of dermatitis induced by TCE.

Apart from the skin disorder, TCE also induced severe liver dysfunction in some patients. For instance, some patients usually had jaundice, liver enlargement, elevated bilirubin, ALT increase and AST increase. In this study, the abnormal values of ALT, AST and T-bilirubin were 91.7%, 83.3% and 66.7%, respectively. During 1990’s to early 2000’s the mortality rate for workers exposed to TCE was around 30% or even higher in TCE-related dermatitis patients in China. However, liver failure, infections, and the resulting sepsis were the principal causes of mortality. A few patients with TCE-induced hypersensitivity dermatitis reportedly died from serious liver dysfunction or from gastrointestinal bleeding along with co-morbidity due to disseminated intravascular coagulation. The liver dysfunction observed in TCE-induced hypersensitivity dermatitis was non-viral and apparently different in its clinical course from usual TCE-induced hepatitis, which occurs without showing rash at elevated TCE exposure concentrations. Since TCE is known to induce serious liver impairment as mentioned above, therefore, the present study sought to investigate the expression of hepatic biotransformation (drug metabolizing enzyme) genes such as CYP1A2, CYP2E1, CYP3A4, CYP2C9 and CYP2A6 in patients that were symptomatic and who had been exposed to TCE.

The toxicity of TCE is dependent on bioactivation, which occurs by two pathways, cytochrome P450 (P450)-dependent oxidation and glutathione (GSH) conjugation. Metabolites of TCE derived from the P450 pathway are associated with the liver.
as a target organ, whereas those derived from the GSH-conjugation pathway are associated with the kidneys\textsuperscript{16} as a target organ. CYP2E1 is the primary P450 enzyme that metabolizes of TCE \textsuperscript{14} and Cummings reported that TCE treatment induced protein expression of rat hepatic CYP2E1 approximately 5-fold and rat renal CYP2E1 approximately 2-fold.\textsuperscript{17} In this study we found that mRNA expression of CYP1A2, CYP2E1, CYP3A4 and CYP2C9 were significantly increased in patients compared with control, indicating that TCE might be the cause of hepatotoxicity to human beings. This finding would seem to exclude the possibility that TCE metabolites exerts a direct effect on liver. However, further studies are needed to reveal whether metabolites have a role in liver injury.

Recently, many studies have addressed the role of the regulatory T cell (Treg) in allergic diseases by preventing disease development via suppressing effector T cell activity such as Th2 cytokine production.\textsuperscript{18,19} Treg has been defined by the specific marker, namely the forkhead box transcription factor (Foxp3) and it was identified as a more specific marker for its roles in maintaining Treg development and function.\textsuperscript{20} These cells have a suppressive effect on inflammatory responses and are recognized as a major cell subset for maintaining peripheral immune tolerance. For instance, the Treg cell prevents both activation and effector function of autoreactive T cells that escaped from other mechanisms of tolerance. They are assumed to control not only pathogenic Th2 cells but also Th1 cells. Additionally, Foxp3 can suppress the expression of T-bet and GATA-3 both with a reciprocal role, that modulates the immune response when increased.\textsuperscript{21,22}

Th1 cells are primary responsible for cell-mediated immunity or delayed-type hypersensitivity (DTH), and Th2 cells for humoral immunity. Nevertheless, it was clear that from the early studies that Th1 cytokine IFN-\(\gamma\) induces the switching of B lymphocytes to several IgG isotypes, and was thus obligatory for some humoral immune responses. The Th1/Th2 polarization and balance are very important in maintaining the normal immunological conditions in humans, and growing evidence suggest that two transcription factors, T-bet and GATA-3, are the determining factors of Th1/Th2 cell differentiation.\textsuperscript{23-25} T-bet plays a key role in Th1 differentiation. It not only controls the expression of the Th1-specific cytokine IFN-\(\gamma\), but also restrains the generation of IL-4 of the Th2-specific cell factor.\textsuperscript{26} Moreover, it also induces the shift of Th2 cell dominance towards the opposite Th1 cell dominance.\textsuperscript{24,27} Basic research as well as clinical studies have shown that Th1 and Th2 cells play a pivotal role in allergen-induced airway inflammation, asthma and allergy,\textsuperscript{28,29} Th1-cell-driven responses seem to be reduced in allergic and asthmatic patients, and balanced
Th1/Th2 cell differentiation is very important for generating appropriate immune responses in antigen exposure. T-bet has been confirmed as a unique Th1-specific transcription factor. On the other hand, the Th2-specific transcription factor, GATA-3, promotes Th2 differentiation and induces Th2 cytokine production in an analogous way to T-bet. The relative expression of T-bet and GATA-3, resulting in a swing in the Th1/Th2 pendulum, has been implicated in a number of immunological diseases, such as asthma, inflammatory bowel diseases, atopic dermatitis, and rheumatoid arthritis. Up-regulated GATA3 mRNA expression level and down-regulated T-bet mRNA transcription level in TCE-exposed patients are changes indicating that TCE exposure might exacerbate skin damage via an influence on the effector T cell differentiation. Cytolytic T lymphocyte-associated antigen-4 (CTLA-4) is a receptor that critically regulates T-cell activation and differentiation by inhibiting cytokine production and cell cycle progression. In vitro CTLA-4 stimulation inhibits Th2 cell differentiation and GATA-3 mRNA expression. In vivo CTLA-4 blockade enhances allergic sensitization and eosinophilic airway inflammation. CTLA-4 knockout mice develop lympho-proliferative disorders characterized by massive CD4^+ T-cell infiltrates with a Th2-skewed phenotype, high IL-4 and IL-5 production. In vivo CTLA-4 blockade has been shown to enhance Th1 cell-mediated diseases such as autoimmune diabetes-2 and experimental autoimmune encephalomyelitis. On the other hand, Th2 cell-mediated responses, such as allergic sensitization and airway inflammation, are also enhanced by CTLA-4 blockade. Ubaldi reported that CTLA-4 inhibits the production of the respective effector cytokines in differentiated Th1 and Th2 cells, confirming its negative role in the control of T-cell effector functions.

Growing evidence has suggested that the balance between Th1 and Th2 immune response is mastered by the upstream transcription factors rather than downstream cytokines production. Thus, the present study focused on the mRNA expression levels of transcription factors including T-bet, GATA-3, Foxp3 and CTLA4 in TCE patients with allergic disorder. Using fluorescent quantitative RT-PCR technology, we tested and compared the mRNA expression patterns of Th1 and Th2 specific transcription factors in peripheral blood lymphocytes, and the result showed that mRNA expression of transcription factors GATA-3, Foxp3 and CTLA4 were significantly elevated in TCE patients more than in healthy controls, whereas, T-bet expression was decreased in TCE patients.

The results cited above indicated that TCE-induced allergic disorder is associated with these immune-related genes and their expression. This might cause a Th1/Th2
imbalance while the up-regulated Treg expression indicating the increased regulatory function of effector T cells. Altogether, TCE might exacerbate skin allergic disorder via an influence on T cell differentiation and function. Furthermore, the current data reconfirmed our previous hypothesis that TCE-induced dermatitis belongs to the allergic reaction disease group and this disorder is a mixed type of immunological disease involving Th1, Th2 and Treg-mediated hypersensitivity.

Regarding the carcinogenic potential of TCE, both laboratory animal tests and epidemiological investigations have indicated that TCE is a potential carcinogen to animals and man. When TCE was given by inhalation for 8 weeks to Sprague-Dawley rats and B6C3F1 mice, it did not produce any effects at 600 and 100 ppm. When given for 104 weeks, TCE caused in rats a dose-related increase in Leydig cell tumors, some non-dose-related increase in the incidence of hemolymphoreticular neoplasias and a low incidence of renal tubuli adenocarcinomas. In Swiss mice, treated for 78 weeks, TCE produced an increase in pulmonary tumors and hepatomas and possibly an increase in the number of total malignant tumors in males. In B6C3F1 mice, TCE caused an increased incidence of the number of total malignant tumors and of pulmonary tumors in females and some increase in hepatomas. In addition, there is substantial evidence in support of an association between TCE exposure and kidney cancer, liver and biliary cancer, non-Hodgkin’s lymphoma as well as more limited evidence of an association with cervical cancer, Hodgkin’s disease and multiple myeloma. However, whether TCE is a potential carcinogen to human beings and its roles in regulating oncogene and apoptotic gene expression remains elusive. As the present study conducted oncogene expression measurements in TCE-induced dermatitis patients, the expression of c-fos, c-myc, k-ras and p53 were impacted to some extent, a result indicating that TCE exposure might increase the incidence of cancer occurrence.

Taken together, our data showed that TCE exposure induced obvious skin reactions in the patients, the hepatic metabolic enzymes including ALT, AST and T-bilirubin were significantly elevated, mRNA expression of T helper cell related genes and apoptosis genes were dramatically changed as well. These results warrant further identification of protein levels of those transcription factors in peripheral blood of TCE-induced hypersensitivity dermatitis, and Th1 and Th2 related cytokine production to further reveal the extent that TCE plays in the progression of skin dermatitis. These findings will improve our understanding of the regulation of T cell activities in the pathogenesis of TCE-induced hypersensitivity dermatitis, a finding that may contribute to future therapeutic strategies of this disorder.
Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References


