Toxicology Letters 288 (2018) 129–135

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Cytokine expression and cytokine-based T-cell profiling in occupational medicamentosa-like dermatitis due to trichloroethylene

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ARTICLE INFO

Keywords:
Trichloroethylene
Cytokines
Transcription factors
$Th_1/Th_2/Th_{17}$/Treg

ABSTRACT

Early diagnosis and treatment of occupational medicamentosa-like dermatitis due to trichloroethylene (OMLDT) are absence of specific and reliable diagnostic/therapeutic biomarkers. This study was conducted on 30 cases of OMLDT, 58 workers exposed to trichloroethylene (TCE) and 40 unexposed controls in order to identify any cytokine signatures that give an index to $CD4^+$T cell differential and serve as biomarkers of OMLDT. Expression profiles of Th_1, Th_2, Th_{17} and Treg cell type-specifying transcription factors and cytokines were analyzed using real time quantitative PCR (RT-qPCR) assay. To explore whether such expression profiles reflected their steady state plasma levels, a Luminex liquid fluorescence analysis was conducted. We found that the expression of transcription factors *FoxP3* transcription factors ($P = 0.006$ and $P < 0.0001$) and *IL-10* cytokine ($P = 0.0008$ and $P < 0.0001$) of the Treg subset were significantly higher in patients than TCE exposure workers and unexposed controls, suggesting that Treg cells were active after the occurrence of OMLDT. The transcript levels of *IL-6* were significantly lower in the TCE exposure groups including patients and exposure workers as compared to the unexposed controls ($P < 0.0001$ and $P = 0.0008$). Circulating levels of assessed cytokines of IL-6 ($P = 0.001$ and $P = 0.011$) and TFN-α ($P = 0.005$ and $P < 0.0001$) were lower in the exposure groups than in the unexposed controls. Compared to the controls, the levels of IL-10 in patients were higher ($P = 0.001$ and $P = 0.0008$). There was a significantly positive correlation between the plasma levels IL-6 and IL-10 in TCE exposed workers. These alterations in the expression of transcription factors and cytokines highlight the underlying dysregulation of T cell subsets in OMLDT that reflect an immune tolerance or immune inhibition. Therefore, the elevation of IL-10 level may be a kind of pathogenesis indicator, and the decline in IL-6 level may be a kind of TCE exposure biomarker. These biomarkers need additional longitudinal follow-up studies to warrant to clinically useful biomarkers of OMLDT.

1. Introduction

Ttrichloroethylene (TCE) contamination is potentially caused by its release into the environment during its manufacture, use and disposal. This includes both industrial pollution and contaminants in ordinary life (Chiu et al., 2006; Rusyn et al., 2014). People exposed to TCE (mostly occupational but sometimes environmental) have been induced to a drug eruption dermatitis (Huang et al., 2015; Seo et al., 2011). Occupational medicamentosa-like dermatitis due to trichloroethylene (OMLDT) shows the clinical diagnostic indicators of skin lesions and mucosal damage, lack of accurate diagnostic and prognostic biomarkers. The main treatment for OMLDT is the rational use of glucocorticoids (Watanabe et al., 2010).

The development of hypersensitivity dermatitis is associated with changes in both differentiation of T helper (Th) cells as well as their cytokine secretion profiles (Pucheu-Haston et al., 2015). pro-inflammatory cytokines and chemokines may contribute to OMLDT and act as immune markers of this inflammation activity (Bassig et al., 2013; Iavicoli et al., 2005; Jia et al., 2012). Most of these cytokines are concentrated on IL-1, IL-6, IL-8, IL-10, IFN-γ and TNF-α which are

Abbreviations: OMLDT, occupational medicamentosa-like dermatitis due to trichloroethylene; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde3-phosphate dehydrogenase; GATA3, GATA-binding protein 3; Treg, regulatory T-cell; Foxp3, Forkhead box P3; T-bet, Th1-specific T box transcription factor; TGF, transforming growth factor; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; Ct, cycle threshold; Th_1, Type 1 helper T cells; Th_2, Type 2 helper T cells; Th_{17}, Type 17 helper T cells; NHL, Non-Hodgkin lymphoma; RORγt, RAR-related orphan receptors; STAT3, Signal transducer and activator of transcription 3

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https://doi.org/10.1016/j.toxlet.2018.02.012
Received 30 September 2017; Received in revised form 31 January 2018; Accepted 8 February 2018
Available online 22 February 2018
0378-4274/

produced by Th_1 and Th_2. Besides the unique genetic alterations representing a signature of TCE exposure (Dai et al., 2015; Shiao, 2009), the special cytokines are optimal candidates in the search for biomarkers of OMLDT.

In this study, we investigated plasma levels of pro- and anti- inflammatory cytokines in patients with OMLDT and the expression profile of characteristic cytokines and transcription factors of Th_1, Th_2, Th_{17} and Treg cells(Gagliani and Huber, 2017). The aim was to dissect the cellular and molecular processes of $CD4^+$T cell differentiation exposed to TCE, and to find the special exposure indicators and incidence indicators.

2. Materials and methods

2.1. Research subjects

According to its clinical manifestations and epidemiological characteristics, OMLDT patients have clear history of TCE exposure. However, there is no dose-effect relationship between the incidence of OMLDT and the contact time and concentration of TCE (Cooper et al., 2009). Therefore, subjects were divided into three groups. The study was conducted at the occupational disease department of the Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, China. The permission to carry out the study was obtained from the director-principal of this institute. The ethical committee clearance was taken from the institutional ethical committee. The study was explained to the faculty members working in the institute and all subjects who agreed to participate in the study were required to sign informed consent. The study was conducted according to Declaration of Helsinki Principles.

A total of 30 patients with OMLDT (PA) (10 females, 20 males) fulfilled the modified 2006 OMDTR criteria for OMLDT (diagnostic criteria of occupational medicamentose-like dermatitis due to trichloroethylene GBZ 185–2006). Individuals with infectious diseases or those who had received immunosuppressive treatment such as irradiation, chemotherapy or glucocorticoid hormones prior to this study were excluded. 40 healthy unexposed controls (UC) (9 females, 31 males) frequency matched by sex and age, with no significant medical problems were also recruited. 58 exposed workers (EW) (19 females, 39 males) came from the simultaneous onset of the patient factory. They had been exposed to the same environment without diseases and had been frequency-matched by sex and age. Demographic characteristics of the three groups are shown in Table 1.

Three milliliters of peripheral venous blood were obtained from participants. A 250 μl aliquot was used for RNA extraction, and the rest was separated to analyze circulating cytokine levels.

2.2. Real-time quantitative reverse transcription (RT-qPCR)

Total RNA was extracted from 250 μl of whole blood using a Rnasey Mini kit (Qiagen, Cat. #74106), then reverse transcribed using a PrimeScript™ RT reagent Kit (TAKARA, Cat. #RR037A) operated in a 30 μl reaction using both random hexamers. The reaction was carried out at 37 °C for 15 min, followed by denaturation at 85 °C for 5s. Reverse transcription cDNA for quantitative detection of fluorescence was also collected. A SYBR green intercalation quantitative PCR (qPCR) was run in an ABI 7500 HT real time PCR system (Applied Biosystems, USA) using custom-designed primer pairs (Sangon Biotech, Shanghai, China) for various cytokines and transcription factors (Table 2). All primers had an amplification efficiency of > 90%. The real-time quantitative PCR system contained 0.4 μl of cDNA, 10 μl 2 × TransStart Tip Green qPCR SuperMix and 0.8 μl primer mix in a 20 μl total reaction volume. The PCR cycling was performed with an initial hold at 94 °C for 30s, followed by 40 cycles of 94 °C for 5 s each, and 60 °C for 30s. A calibration curve was constructed by plotting the PCR threshold cycle (Ct) value against the ln (template dilution). The relative mRNA expression levels in the experimental samples were calculated using the $2^{-\Delta\Delta Ct}$ method with the standard curve, and normalized to GAPDH mRNA levels.

Table 1
Demographic and clinical profile of study subjects.

Characteristic	PA(n = 30)	EW(n = 58)	UC(n = 40)	F/χ^2value
Mean age (SD)	27.14 ± 8.32	27.25 ± 8.91	29.88 ± 6.43	5.069[a]
Sex (n, %)				
Male	20 (67)	39 (67)	31 (78)	1.44[b]
Female	10 (33)	19 (33)	9 (23)	

Note: PA (OMLDT patients); EW (exposed workers); UC (unexposed controls).
[a]ANOVA test.
[b]Pearson χ^2 test.
*$P < 0.05$.

2.3. Cytokine production assays

A MILLIPLEX® MAP Human Th_{17} magnetic bead panel kit (Millipore Corporation, Cat. #HTH17MAG–14 K, USA) was used to quantify IFN-γ, IL-1β, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-17A, IL-17F, IL-13, IL-22 and IL-23 cytokines in plasma samples following manufacturer's instructions. After reconstitute Quality Controls and Standard, the gradient dilution of Standard was carried out in sequence. The Quality Controls, serial standards and plasma samples were added into 96-well plates. The Mixed Beads and Detection Antibodies were added successively into each well. After incubation Mean Fluorescent Intensity (MFI) data of 96 wells of plates were collected by the MILLIPLEX® MAGPIX System (Millipore Corporation, USA) and analyzed using MILLIPLEX Analyst v.5.1 and 5-parameters logistic curve-fitting way for calculating cytokine/chemokine concentrations (pg/ml) (Fig. 3).

2.4. Statistical analyses

Comparisons of the subjects' demographic characteristics used one-way analysis of variance (ANOVA) or Pearson χ^2. A Kolmogorov-Smirnov assay was used to check the normal distribution of observation dates, and Levene's test was performed to analyze the homoscedasticity between groups. Then, the statistical analyses of cytokine levels and gene expression levels were compared with the three groups using the two-tailed nonparametric Kruskal-Wallis test. Meanwhile, a Mann–Whitney *U* test was used to find the difference between the two groups and the Bonferroni adjusted *P*-value. The two-tailed, nonparametric Spearman correlation test was executed among the cytokine levels in three groups. A *P*-value of less than 0.05 was considered significant. Results were analyzed with SSPS 19.0 (IBM, USA), and drawn with Graph Pad Prism 6.0 (http://www.graphpad.com/).

3. Results

3.1. The expression of transcription factor and cytokine genes in peripheral blood

The expressions of special genes encoding characteristic transcription factors of Th_1, Th_2, Th_{17} and Treg subsets were analyzed among the three groups (Fig. 1).The expressions of transcription factors *T-bet* (Th_1) and *GATA3* (Th_2) were significantly higher in the EW and PA groups than in the UC group (*T-bet*: $P < 0.0001$, $P < 0.0001$; *GATA3*: $P < 0.0001$, $P = 0.02$). There was a significantly higher expression of *FoxP3* (Treg) in the PA group than in the EW and UC groups ($P = 0.006$, $P < 0.0001$). The expressions of *STAT3* and *RoR-γt* (Th_{17}) showed no statistical difference between the three subject groups ($P = 0.069$, 0.63).

The expressions of various genes of cytokines as well as pro- or anti-