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Occupational exposure to trichloroethylene is associated with a decline in lymphocyte subsets and soluble CD27 and CD30 markers

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Occupational cohort and case–control studies suggest that trichloroethylene (TCE) exposure may be associated with non-Hodgkin lymphoma (NHL) but findings are not consistent. There is a need for mechanistic studies to evaluate the biologic plausibility of this association. We carried out a cross-sectional molecular epidemiology study of 80 healthy workers that used TCE and 96 comparable unexposed controls in Guangdong, China. Personal exposure measurements were taken over a three-week period before blood collection. Ninety-six percent of workers were exposed to TCE below the current US Occupational Safety and Health Administration Permissible Exposure Limit (100 p.p.m. 8 h time-weighted average), with a mean (SD) of 22.2 (36.0) p.p.m. The total lymphocyte count and each of the major lymphocyte subsets including CD4+ T cells, CD8+ T cells, natural killer (NK) cells and B cells were significantly decreased among the TCE-exposed workers compared with controls ($P < 0.05$), with evidence of a dose-dependent decline. Further, there was a striking 61% decline in sCD27 plasma level and a 34% decline in sCD30 plasma level among TCE-exposed workers compared with controls. This is the first report that TCE exposure under the current Occupational Safety and Health Administration workplace standard is associated with a decline in all major lymphocyte subsets and sCD27 and sCD30, which play an important role in regulating cellular activity in subsets of T, B and NK cells and are associated with lymphocyte activation. Given that altered immunity is an established risk factor for NHL, these results add to the biologic plausibility that TCE is a possible lymphomagen.

Abbreviations: NK, natural killer; NHL, non-Hodgkin's lymphoma; TCE, trichloroethylene.

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Introduction

Trichloroethylene (TCE) is a volatile chlorinated organic compound and commonly used in industrial settings as a degreaser for metal parts and general-purpose solvent for lipophilic compounds. In 1995, it was estimated that >400 000 workers were exposed to TCE on an annual basis in the USA (1). Further, as a consequence of its presence in workplaces for many years, TCE has become a widespread environmental water contaminant. Approximately 34% of the drinking water supplies that have been tested in the USA had some level of TCE contamination, and it was reported that ∼10% of the USA population had detectable levels of TCE in their blood (2,3).

TCE is a rodent carcinogen (4) but its carcinogenicity in humans is unclear. There is a voluminous experimental literature on short-term biologic effects of TCE exposure and recent studies indicate that TCE alters key lymphocyte subset levels, particularly $CD4^+$ T cell populations, and inhibits activation-induced cell death of $CD4^+$ T cells (5–7). There is some evidence of an association with non-Hodgkin lymphoma (NHL) in epidemiological studies but findings are not entirely consistent (4,8–11). The 1995 International Agency for Research on Cancer review of TCE classified TCE as a probable (Group 2A) human carcinogen based on sufficient evidence of TCE's carcinogenicity in animals and limited evidence of TCE's carcinogenicity in humans, with elevated risks suggested for liver and biliary tract cancer and NHL (4,11). However, the carcinogenicity of TCE and its regulation is a matter of continuing debate despite an extensive database of *in vitro* and *in vivo* animal studies and several cohort and case–control studies.

Wartenberg *et al.* (11) noted that few epidemiologic studies of TCE carcinogenicity have been able to evaluate the isolated effects of TCE and recommended that biomarker studies of populations with isolated exposure to TCE be carried out. The few published cross-sectional biomarker studies of TCE-exposed workers are relatively small and have serious limitations with regard to evaluation of potential confounding exposures. To address questions about TCE's potential mechanism of action in humans, we carried out a cross-sectional study to evaluate the impact of occupational exposure to TCE on peripheral blood cells including lymphocyte subset counts and soluble CD27 and CD30, two members of the tumor necrosis factor receptor superfamily that play an important role in regulating cellular activity in subsets of T, B and natural killer (NK) cells (12–14).

Methods

Study design

To select factories for study, we conducted an initial screening of >40 potential study factories over a one-year period using Dräger tubes and 3 M badges to measure TCE and other chemicals including benzene, styrene, ethylene oxide, formaldehyde, methylene chloride, chloroform, perchloroethylene and epichlorohydrin. Factories were included if they used TCE in manufacturing processes, had no detectable benzene, styrene, ethylene oxide, formaldehyde or epichlorohydrin levels, and low to negligible levels of other chlorinated solvents. Duplicate badges for TCE were analyzed in Guangdong and the USA and showed similar results with a Pearson correlation of 0.99. Ultimately, six study factories with metal (n = 4), optical lense (n = 1) and circuit board (n = 1) cleaning processes were identified that fulfilled the above selection criteria.

In June and July 2006, we carried out a cross-sectional study of 80 workers currently exposed to TCE in the six study factories with TCE cleaning operations and 96 unexposed controls. Control workers were enrolled from two clothes manufacturing factories, one food production factory and a hospital that did not use TCE and were in the same geographic region as the factories that used TCE. Controls were frequency-matched by sex and age (±5 years) to exposed workers. Exclusion criteria for both TCE-exposed and control workers were history of cancer, chemotherapy and radiotherapy, as well as previous occupations with notable exposure to benzene, butadiene, styrene and/or ionizing radiation. The study was approved by Institutional Review Boards at the US

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National Cancer Institute and the Guangdong National Poison Control Center, China. Participation was voluntary and all subjects gave written informed consent.

Exposure measurement and sample collection

Full-shift personal air exposure measurements, two to three per subject, were taken in a three-week time-period in the factories using 3 M organic vapor monitoring badges before blood collection. All samples were analyzed for TCE and a subset (48 from TCE-exposed workers) was analyzed for a panel of organic hydrocarbons including benzene, methylene chloride, perchloroethylene and epichlorohydrin. In two of the metal degreasing factories, workers were intermittently using respirators. Only one subject wore gloves while cleaning with TCE. Organic vapor monitoring samples were obtained on a subgroup of control workers in the food and clothes production factories. Subjects were interviewed using a questionnaire that requested information about demographic and lifestyle characteristics and occupational history. They were also asked to provide a 29 ml peripheral blood sample, buccal cell mouth rinse sample, post-shift and overnight urine samples and undergo a brief physical exam that included measurement of blood pressure, height, weight and temperature and evaluation for signs of current upper or lower respiratory infection.

Assays

Blood samples were delivered to the laboratory within 6 h of being collected, where the complete blood count and differential and major lymphocyte subsets were analyzed on the same day that a peripheral blood sample was collected. Plasma sCD27 and sCD30 were measured in duplicate by an enzyme-linked immuosorbent assay (Bender Medsystems, Vienna, Austria). Exposed workers and controls were assayed consecutively within the same batch. Measurements from blinded quality control replicates interspersed among the samples did not identify outlier batches. Assay coefficient of variations were <10% for each parameter from the complete blood count and lymphocyte subset analysis and were 31 and 32% for sCD27 and sCD30, respectively. Normal ranges for peripheral blood cells are listed in supplementary Table 1 (available at *Carcinogenesis* Online).

Statistical analysis

Unadjusted summary measures are presented for all end points. Linear regression using the natural logarithm (ln) of each end point was used to test for differences between control and exposed workers and to evaluate for a dose–response across exposure groups, i.e. controls, workers exposed to <12 p.p.m. TCE (the median exposure level) and ≥12 p.p.m. TCE. TCE air levels were based on the arithmetic mean of an average of two to three measurements per subject. All statistical models included the frequency matching factors age (as a continuous variable) and sex. In addition, potential confounders that have been shown previously to influence one or more of the end points in this report were included in a model for a given end point if the regression coefficient was altered by ±15% and included current cigarette smoking status (yes/no), current alcohol consumption (yes/no), recent infections (flu or respiratory infections in the previous month) and body mass index. The total lymphocyte percent from the complete blood count was used to calculate three lymphocyte subsets (i.e. $CD4^+$ T cells, $CD8^+$ T cells and NK cells), and an additional calculation was carried out using the lymphocyte percent obtained by flow cytometry and compared for quality control purposes. If there was more than a 15% SD between the two measures, then the samples were deleted for the analysis of lymphocyte subsets (i.e. three samples, 1.7% of data). All analyses were carried out using SAS version 9.0 software (SAS Institute, Cary, North Carolina).

Results

Subjects were categorized into three groups by mean TCE levels measured during the month before phlebotomy [controls, <12 p.p.m. (mean = 5 p.p.m.) and ≥12 p.p.m. (mean = 38 p.p.m.)]. Demographic characteristics including age, sex distribution, current smoking status and body mass index were comparable among the three groups (Table I). TCE exposure was negligible in the control factories.

The total lymphocyte count and all major lymphocyte subsets including $CD4^+$ T cells, $CD8^+$ T cells, NK cells and B cells were statistically significantly lower in workers exposed to TCE than controls, and there was a significant dose–response relationship across the categories of controls and lower- and higher-exposed workers (Figure 1a and b; supplementary Table 2 is available at *Carcinogenesis* Online). In contrast, there was no evidence that TCE exposure influenced the granulocyte, platelet or monocyte counts (Figure 1a, supplementary Table 2 is available at *Carcinogenesis* Online). There was a small decline in plasma hemoglobin concentration but no difference in the red blood cell mean corpuscular volume or platelet counts (supplementary Table 2 is available at *Carcinogenesis* Online). There was a substantial decline in sCD30 and a striking decline in sCD27 (i.e. 61%) plasma levels in TCE-exposed individuals compared with controls (Figure 2), and workers exposed to even relatively low levels of TCE (<12 p.p.m.) had significantly lower levels of both compounds (supplementary Table 2 is available at *Carcinogenesis* Online).

To evaluate the influence of exposure to other chlorinated solvents that were present at relatively low levels in some factories, we excluded one or more of these factories at a time from the analyses and found that results were similar and conclusions unchanged (data not shown). We repeated the analyses in the 77 (of a total of 80) workers exposed to <100 p.p.m. TCE, the current Occupational Safety and Health Administration Permissible Exposure Limit (http://www.osha

Table I. Demographic characteristics and TCE exposure level

Subjects	Controls (n = 96)	Exposed		
		Total (n = 80)	<12 p.p.m. (n = 39)	≥12 ppm (n = 41)
Demographic characteristics				
Age, mean (SD)[a]	27 (7)	25 (7)	24 (5)	27 (8)
BMI, mean (SD)[a]	22 (3)	21 (3)	21 (2)	22 (3)
Sex[b], n (%)				
Female	23 (24)	23 (29)	15 (38)	8 (20)
Male	73 (76)	57 (71)	24 (62)	33 (80)
Current smoke[b], n (%)				
No	58 (60)	46 (58)	22 (56)	24 (59)
Yes	38 (40)	34 (42)	17 (44)	17 (41)
Current alcohol use[b], n (%)				
No	56 (58)	54 (68)	26 (67)	28 (68)
Yes	40 (42)	26 (32)	13 (33)	13 (32)
Recent infection[b], n (%)				
No	75 (78)	65 (81)	31 (79)	34 (83)
Yes	21 (22)	15 (19)	8 (21)	7 (17)
TCE exposure				
TCE air level (p.p.m.)[c], mean (SD)	<0.03	22.19 (35.94)	5.19 (3.47)	38.36 (44.61)

BMI, body mass index.
[a]Mean ± standard deviation.
[b]Number (percent).
[c]TCE air level is the arithmetic mean (±SD) of an average of two measurements per subject collected during the month before phlebotomy.

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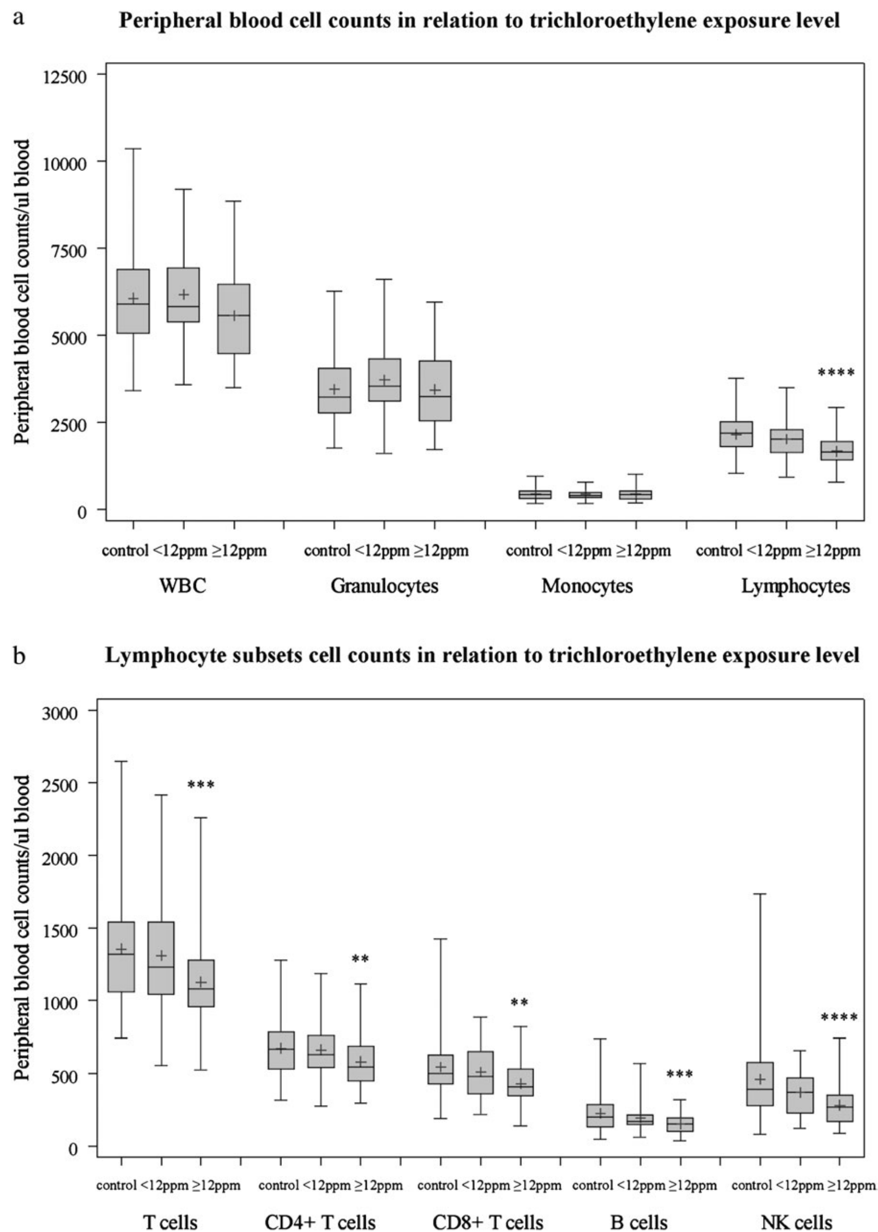

Fig. 1. (**a**) Peripheral blood cell counts in relation to TCE exposure level. P_{trend} using category of TCE levels (controls, <12 p.p.m. and ≥ 12 p.p.m.) as a continuous variable. The median TCE concentration of all exposed subjects was 12 p.p.m. Differences in cell counts were tested by linear regression analysis of ln-transformed end point, adjusting for relevant covariates [white blood cell (WBC): adjusted for age, sex, smoking status and body mass index; granulocytes: adjusted for age, sex and body mass index; Monocytes: adjusted for age, sex and smoking status; lymphocyte: adjusted for age and sex]. (**b**) Lymphocyte subsets cell counts in relation to TCE exposure level. Differences in cell counts were tested by linear regression analysis of ln-transformed end point, adjusting for relevant covariates [$CD4^+$, $CD8^+$ and NK T cells: adjusted for age and sex, three subjects (two controls and one exposed) were deleted due to inconsistent cell counts using complete blood count data versus flow cytometry to calculate % lymphocytes; B cell: adjusted for age, sex and smoking status]. The P values are indicated as: $^{**}P < 0.01$; $^{***}P < 0.001$ and $^{****}P < 0.0001$.

.gov/dts/chemicalsampling/data/CH_273000.html) and found that the strength of the associations shown in Figures 1a and b and 2 were essentially unchanged. Further, we carried out analyses among the 60 workers exposed to <25 p.p.m. TCE, which is the current National Institute of Occupational Safety and Health Recommended Exposure Limit and found that the total lymphocyte count, all lymphocyte subsets and sCD27 and sCD30 concentrations were still significantly lower at $P < 0.05$ compared with controls.

Discussion

We found that exposure to relatively low levels of TCE, including at levels lower than the Occupational Safety and Health Administration Permissible Exposure Limit and National Institute of Occupational Safety and Health Recommended Exposure Limit, was associated with a decline in total peripheral blood lymphocytes and all major lymphocyte subsets, including $CD4^+$ T cells, which is consistent with experimental reports (6,7). Further, we showed that TCE exposure was also associated with a decline in sCD27 and sCD30 and that sCD27 levels appeared to be particularly sensitive to TCE exposure.

TCE exposure has been associated with immunosuppression in animals (6,15). In humans, there are little data on immunosuppression from TCE exposure (6,11,16). There is some evidence that exposure to TCE in humans is associated with alterations in levels of certain cytokines (12–14,17). There is also some evidence that TCE exposure is associated with several autoimmune diseases in humans such as

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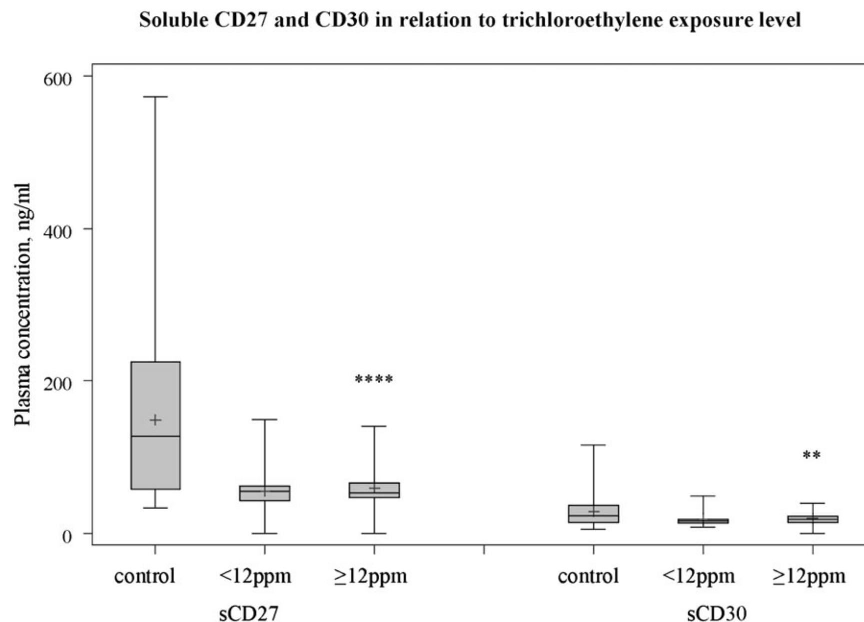

Fig. 2. Soluble CD27 and CD30 in relation to TCE exposure level. P_{trend} using category of TCE levels (controls, <12 p.p.m. and ≥12 p.p.m.) as a continuous variable. The median TCE concentration of all exposed subjects was 12 p.p.m. Differences in sCD27 and sCD30 were tested by linear regression analysis of ln-transformed end point, adjusting for age, sex and infection. For sCD27, results available for 38 subjects exposed to <12 p.p.m. TCE. The *P* values are indicated as: $^{**}P < 0.01$; and $^{****}P < 0.0001$.

systemic sclerosis, systemic lupus erythematosis, rheumatoid arthritis and dermatitis (6,11). Our data show that TCE exposure was associated with decline in all major lymphocyte subsets tested and with important markers of lymphocyte function and activation, sCD27 and sCD30, which can play a key role in maintaining control of T-cell regulation (12–14). The fact that we see strong effects on lymphocytic parameters and no effect on granulocytes, platelets or monocytes suggests that there is a relatively selective effect on lymphoid progenitors or on the lymphocyte maturation process, rather than a broad impact on stem cells in the bone marrow. These results contrast with the pattern we and others have observed among humans exposed to benzene, i.e. a strong and broad effect across all blood cell types that probably results from suppression of bone marrow stem or progenitor cell development (18).

Both sCD27 and sCD30 shed by B and T cells at activation are important co-stimulator molecules in the regulation of the balance between Th1 and Th2 responses (12,14). They have been found to be altered in patients with a variety of immunopathological diseases and have been used as markers to monitor immune activation as well as to assess therapeutic effects of immunosuppressive and antiviral treatment (19–22). There is a possibility that the effects we see for sCD27 and sCD30 and TCE exposure could simply be a function of suppressed lymphocyte counts. However, we did not observe a correlation between lymphocyte subsets and sCD27 and sCD30 in TCE-exposed workers (data not shown), consistent with other report (23).

We observed a decrease in lymphocyte subsets and a substantial decline in markers of lymphocyte activation. Given that altered immunity including immunosupression is an established risk factor for NHL, these results add to the biologic plausibility that TCE is a lymphomagen (24). More specifically, a decrease in CD4+ T cells has been associated with an increased future risk of NHL in HIV-infected populations (25), in post-renal transplantation patients who receive immunosuppressive therapy (26) and in patients with Sjogren's syndrome (27). As noted, both sCD27 and sCD30 are considered markers of lymphocyte activation. Decreased levels of sCD27 following chemotherapy and bone marrow transplantation have been reported, consistent with an immunosuppressive state induced by certain regimens (28,29).

Our study is the first investigation of the impact that TCE exposure has on lymphocyte subsets, sCD27 and sCD30. We report that TCE was associated with a decrease in all major lymphocyte subsets (i.e. $CD4^+$ T cells, $CD8^+$ T cells, NK cells and B cells) and soluble CD27 and CD30 among workers exposed to TCE under the current Occupational Safety and Health Administration workplace standard. These results, if confirmed in future studies, show that TCE exposure broadly affects several components of the immune system and provide additional support for the biologic plausibility that TCE is associated with risk of NHL.

Supplementary material

Supplementary Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

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