Circulating Soluble CD27 and CD30 in Workers Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

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Abstract

Previous studies suggest that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure may be associated with non-Hodgkin lymphoma (NHL) but findings remain inconclusive. There is a need for mechanistic studies to evaluate the biologic plausibility of this association. In this cross-sectional study, we investigated changes in plasma levels of interleukin 1 receptor antagonist (IL1RA) and soluble (s)CD27 and sCD30 which have been found to be predictive of lymphoma, among workers of a cohort occupationally exposed to TCDD. Eighty-five workers who had been exposed to TCDD more than 30 years before blood collection were included in the current investigation. Plasma level of the markers was measured by ELISA. Current plasma levels of TCDD were determined by high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry. TCDD blood levels at time of last exposure were estimated using a one-compartment first order kinetic model. Exposure-response analyses showed no significant association between blood levels of sCD27, and sCD30 and current and estimated TCDD levels at time of last exposure. IL1RA showed a borderline significant decrease with increasing plasma TCDD levels ($P = 0.07$), which reached formal statistical significance when excluding subjects with chronic diseases. In conclusion, no clear dose–response relationship was observed between the measured markers and TCDD level. However, there was a suggestion that markers in particular IL1RA tended to decrease with increasing TCDD levels. This observation is consistent with our earlier observation on decreasing cytokine levels, suggesting immunosuppression, with increasing exposures. These findings possibly provide new insights in the etiology of NHL and the mechanisms through which TCDD can increase lymphoma risk. *Cancer Epidemiol Biomarkers Prev; 22(12); 1–5. ©2013 AACR.*

Introduction

Recent prospective studies have shown a possible association between blood levels of selected cytokines and small cell-signaling protein molecules and future risk of non-Hodgkin lymphoma (NHL; refs. 1–6). Most notably soluble(s) CD30 has been found to be associated to lymphoma in three prospective studies (1, 3, 4). In addition sCD27 has been associated with lymphoma in the largest prospective studies to date (1, 3). Interleukin 1 receptor antagonist (IL1RA) has been linked to lymphoma risk in genetic studies (7) and in experimental and case–control studies (8, 9). As the immune system plays a pivotal role in

lymphomagenesis, it is hypothesized that risk factors of lymphoma such as environmental and occupational exposures operate through deregulation of the immune system, which may be reflected by perturbations in these prediagnostic markers.

Exposure to phenoxy herbicides, which are often contaminated with dioxins such as 2,3,7,8-tetrachlorodibenzo-p dioxin (TCDD), has been linked to several cancers among which NHL (10–13). However, few epidemiologic studies have evaluated the possible effects of TCDD on markers of the immune system among which cytokines and chemokines. These studies have provided some evidence for a downregulation of the immune system but evidence is still limited (14–17). To date, no study has examined effects of TCDD exposure on blood soluble immune markers.

In our previous study (17), we showed that plasma levels of a large panel of cytokines, chemokines, and growth factors among workers occupationally exposed to TCDD tended to show a decrease with increasing TCDD levels. Here, we extend these analyses to the evaluation of TCDD exposure on blood levels of IL1RA and soluble markers of sCD27 and sCD30, two members of the tumor necrosis factor (TNF) receptor superfamily that play an important role in regulating cellular activity in subsets of T, B, and natural killer cells. Study subjects

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comprised a subset of a retrospective cohort of Dutch workers from two chlorophenoxy herbicide producing factories, part of the IARC multinational study of workers exposed to chlorophenoxy herbicides, chlorophenols, and dioxins during their working life (10, 18, 19).

Materials and Methods

Study population

A detailed description of the cohort study design and exposure assessment can be found elsewhere (18, 19). Current analyses utilized a subset of workers from one factory (labeled "A" in previous publications) who were exposed to TCDD as a byproduct of production of 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol during 1953 to 1969, and/or during an occupational accident in 1963 (17). Subjects were selected for blood collection based on stratified sampling of (assumed) high-exposed and low-exposed workers. A-priori exposure status was based on a detailed occupational history including periods of employment in different departments and positions held and a detailed industrial hygiene evaluation of jobs and departments (17). Fifty high-exposed workers who had been exposed due to the industrial accident (both factory workers and contract workers involved in the clean-up after the accident) or working in departments of main production and 43 low-exposed workers employed in non-production departments who were matched by factory, sex, age, and current residence were selected for blood collection. All study subjects were male. Written informed consent was obtained from each study subject after the study was explained. Participants were asked to complete a self-administered questionnaire, which included questions on occupational and medical history, lifestyle factors and anthropometric characteristics (17, 19).

Blood markers measurements

Plasma level of sCD27, sCD30, and IL1RA was measured by ELISA (Bender Medsystems: BMS286INST, BMS240, and BMS2080 kits respectively). A subset of samples ($n = 68$; 34 high-exposed and 34 low-exposed) was analyzed in duplicate. The median coefficient of variation was 7.01%, 4.05%, and 7.14% for sCD30, sCD27 and IL1RA respectively. Intraclass of correlation coefficients (ICC) was 0.95, 0.99, and 0.99 for sCD30, sCD27, and IL1RA, respectively. IL1RA measurement was below the limit of detection for one subject and this value was imputed based on $min/\sqrt{2}$.

Exposure measurements

Heparin plasma samples were analyzed for TCDD, at the Centers for Diseases Control and Prevention (CDC) using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry. As we measured current levels of TCDD (TCDD$_{current}$) approximately 35 years since last exposure (lag) and TCDD is highly persistent with a long half-life in blood and human tissues, a one-compartment first-order kinetic model with a TCDD half-life ($t_{1/2}$) of 7.1 years was used to estimate TCDD blood levels at the time of last exposure (TCDD$_{max}$; refs. 17, 19). Current TCDD levels and estimated maximum TCDD levels were subsequently used to investigate exposure-response relations between TCDD levels and sCD27, sCD30, and IL1RA. Individual TCDD levels (high-exposed, $n = 15$; low-exposed, $n = 20$), which were below the limit of detection, were imputed using a maximum likelihood estimation method as previously described (19).

Statistical analysis

The two-sample t test was used to test for statistical significance of differences in the blood marker concentrations between high- and low-exposed subjects. Linear regression models adjusted for potential confounders, i.e., body mass index, alcohol intake, smoking status, medication, and chronic and acute medical conditions were used to explore exposure–response relations between sCD27, sCD30 and ILRA and TCDD$_{current}$ or TCDD$_{max}$. Moreover, exposure–response relations were also investigated using subjects free of chronic disease at the time of blood draw as a sensitivity analysis as previous analyses showed that subjects with chronic disease unduly influenced the analyses. All P values were two-sided, with $P < 0.05$ considered statistically significant.

Results

After excluding two subjects with missing TCDD results and 6 subjects with a previous (non-skin) cancer diagnosis, a total of 85 subjects were available for analysis. High- ($n = 47$) and low-exposed workers ($n = 38$) had identical distributions of covariate variables (Table 1) except a significant higher alcohol consumption in low-exposed workers compared with high-exposed workers ($P = 0.05$). Geometric mean of TCDD$_{current}$ and TCDD$_{max}$ were significantly higher in high-exposed workers (TCDD$_{current}$: 3.25 ± 7.43 ppt; TCDD$_{max}$: 79.82 ± 33.28 ppt) compared with low-exposed workers (TCDD$_{current}$: 1.07 ± 6.42 ppt; TCDD$_{max}$: 7.53 ± 32.14 ppt). Blood concentration of all markers did not significantly differ between the two exposure groups although marker levels tended to be lower in high-exposed workers as compared to low-exposed workers (Table 2). We found no significant association between blood levels of sCD27, sCD30, and IL1RA and TCDD$_{current}$ (Table 2) or TCDD$_{max}$ (data not shown) in the confounder adjusted linear regression analyses; although as previous indicated a tendency of lower marker levels were found. Sensitivity analyses based on subjects without chronic disease ($n = 41$) showed significant association for IL1RA [Estimate $= -0.11$, 95% confidence interval (CI) $= -0.183$–0.038; $P = 0.004$, adjusted for age, BMI, and alcohol). However, due to small sample size, this finding should be interpreted with caution.

Table 1. General characteristics of high and low TCDD-exposed workers

	High-exposed ($n = 47$)	Low-exposed ($n = 38$)	P-value[f]
Age, y[a]	69.07 (7.45)	68.55 (7.93)	0.75
Body mass index (kg/m^2)[a]	27.33 (3.10)	26.65 (3.04)	0.31
Alcohol intake (units/week)[a]	11.48 (13.56)	17.26 (13.16)	0.05
Smoking status, N (%)			0.84
Current smoker	12 (25.5%)	8 (21.1%)	
Former smoker	28 (59.6)	23 (60.5%)	
Never smoker	7 (14.9%)	7 (18.4%)	
Skin cancer, N (%)	4 (8.5%)	3 (7.9%)	0.92
Infectious disease in the past 4 weeks, N (%)	4 (8.7%)	4 (10.5%)	0.78
Chronic disease, N (%)[b]	24 (51.1%)	20 (52.6%)	0.89
Chronic inflammatory disease, N (%)[c]	13 (27.7%)	11 (28.9%)	0.90
Medication, N (%)			0.33
Immunosuppressant	5 (10.6%)	4 (10.5%)	
NSAIDs	14 (29.8%)	6 (15.8%)	
Antibiotics	0	1 (2.6%)	
TCDD$_{current}$ (ppt)[d]	3.25 (7.43)	1.07 (6.42)	0.002
TCDD$_{max}$ (ppt)[e]	79.82 (33.28)	7.53 (32.14)	0.001

Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.
[a]Mean (SD).
[b]Chronic diseases included: diabetes, coronary heart disease, and hypertension.
[c]Chronic inflammatory diseases: chronic obstructive pulmonary disease, psoriasis, sarcoidosis, asthmatic bronchitis, rheumatoid arthritis, liver failure, Crohn's disease, fibromyalgia, and allergy.
[d]Current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD$_{current}$) parts per trillion, geometric mean (geometric standard deviation).
[e]Estimated maximum levels of TCDD (TCDD$_{max}$) parts per trillion, geometric mean (geometric standard deviation).
[f]P values from t tests for continuous variables and χ^2 tests for categorical variables.

Discussion

Both sCD27 and sCD30, members of the TNF receptor superfamily, are considered markers of lymphocyte activation (20, 21). Studies have shown that levels of sCD27 and sCD30 decreased following chemotherapy, which induces an immunosuppressive status (22–24). The observed general reduction in blood levels of measured markers with increasing TCDD levels is consistent with the earlier observation among the same study subjects that indicated a general decrease in most cytokine-, chemokine-, and growth factors levels with increasing TCDD exposure levels (17) and provides more evidence that TCDD exposure could lead to a general suppression of the immune system. Therefore, given that immunosuppression is an established risk factor for NHL (25) and given the suggested (animal, experimental, and human)

Table 2. Mean blood marker concentrations for the low and high TCDD-exposed groups and the linear estimates of the association between blood markers and TCDD$_{current}$ levels

	High-exposed ($n = 47$)	Low-exposed ($n = 38$)		Multivariate linear model[b]	
	GM (GSD)	GM (GSD)	P[a]	Estimates	95% CI
sCD30	23.95 (1.51)	24.55 (1.43)	0.77	−0.034	−0.083–0.014
sCD27	72.07 (2.11)	74.49 (1.40)	0.80	−0.053	−0.129–0.023
IL1RA	458.98 (1.88)	473.76 (1.93)	0.82	−0.074	−0.152–0.003

Abbreviations: GM, geometric mean; GSD, geometric standard deviation.
[a]P values are from t tests of natural log-transformed values.
[b]Covariates included in the multivariate models were age, body mass index, alcohol intake, smoking, chronic disease, chronic inflammatory disease, infectious disease within 4 weeks before blood sampling, and medication. Linear estimates are based on log-transformed values of blood markers and TCDD measurements.

evidence for an association between TCDD exposure and immune suppression (26, 27), these results support the biologic plausibility that TCDD could be involved in the development of lymphomas and might explain the observed increased NHL risk in this cohort of workers exposed to chlorophenoxy herbicides, chlorophenols, and contaminants (19).

We found a significant negative association between IL1RA and TCDD level among workers without chronic disease. There were no significant differences between workers with and without chronic diseases in TCDD exposure levels and baseline characteristics except that workers with chronic disease used more anti-inflammatory drugs (22.4% compared with 1.2%). The higher use of inflammatory drugs among the subjects with chronic diseases may have masked the observed IL1RA-TCDD association that was observed among the subjects without a chronic disease. IL1RA has been shown to play a crucial role in the prevention of various inflammatory diseases and counteract inflammatory effects of IL1 family members (28). Moreover, chronic inflammation has been linked to several cancers including NHL, gastric cancer, hepatocellular carcinoma, and adenocarcinoma of the large intestine (29). A recent genetic study found *IL1RN* to be associated with NHL risk (7). *IL1RN* is known to alter IL1β expression levels (30), leading to a modulation of a variety of IL1–related immune and inflammatory responses. In addition, a case–control study showed that pretreatment serum level of IL1RA was associated to increased risk of diffuse large B-cell lymphoma and follicular lymphoma (9). However, in a recent nested case-control study examining IL1RA in prediagnosed blood samples, no association was found between IL1RA and NHL (6). However, taken together, it would suggest that inflammatory pathways may be another relevant mechanism for the association between TCDD exposure and NHL development.

Our study is the first investigation that examined the effect of TCDD exposure on blood markers of sCD27, sCD30, and IL1RA in human, markers that have been linked to lymphoma risk (1–4, 7–9). Together with our previous study on immune markers the results suggest a decrease in several components of the immune system in relation to TCDD exposure.

References

1. De Roos AJ, Mirick DK, Edlefsen KL, LaCroix AZ, Kopecky KJ, Madeleine MM, et al. Markers of B-cell activation in relation to risk of non-hodgkin lymphoma. Caner Res 2012;72:4733–43.
2. Purdue MP, Lan Q, Bagni R, Hocking WG, Baris D, Reding DJ, et al. Prediagnostic serum levels of cytokines and other immune markers and risk of non-hodgkin lymphoma. Cancer Res 2011;71:4898–907.
3. Vermeulen R, Saberi Hosnijeh F, Portengen L, Krogh V, Palli D, Panico S, et al. Circulating soluble CD30 and future risk of lymphoma; evidence from two prospective studies in the general population. Cancer Epidemiol Biomarkers Prev 2011;20:1925–7.
4. Purdue MP, Lan Q, Martinez-Maza O, Oken MM, Hocking W, Huang WY, et al. A prospective study of serum soluble CD30 concentration and risk of non-Hodgkin lymphoma. Blood 2009;114:2730–32.
5. Saberi Hosnijeh F, Krop EJM, Scoccianti C, Krogh V, Palli D, Panico S, et al. Plasma cytokines and future risk of non-hodgkin lymphoma (NHL): a case-control study nested in the Italian European Prospective Investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev 2010;19:1577–84.
6. Gu Y, Shore RE, Arslan AA, Koenig KL, Liu M, Ibrahim S, et al. Circulating cytokines and risk of B-cell non-Hodgkin lymphoma: a prospective study. Cancer Causes Control 2010;21:1323–33.
7. Hosgood HD III, Purdue MP, Wang SS, Zheng T, Morton LM, Lan Q, et al. A pooled analysis of three studies evaluating genetic variation in innate immunity genes and non-Hodgkin lymphoma risk. Br J Haematol 2011;152:721–6.
8. Shin SS, Traweek ST, Ulich TR. Expression of interleukin-1 receptor antagonist protein in malignant lymphomas: an immunohistochemical study. Arch Pathol Lab Med 1995;119:247–51.
9. Charbonneau B, Maurer MJ, Ansell SM, Slager SL, Fredericksen ZS, Ziesmer SC, et al. Pretreatment circulating serum cytokines associated with follicular and diffuse large B-cell lymphoma: A clinic-based case-control study. Cytokine 2012;60:882–9
10. Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesqurta HB, et al. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins an expanded and updated international cohort study. Am J Epidemiol 1997;12:1061–75.
11. Hardell L, Lindström G, van Bavel B, Fredrikson M, Liljegren G. Some aspects of the etiology of non-Hodgkin's lymphoma. Environ Health Perspect 1998;106(Suppl 2):679–81.
12. Bodner KM, Collins JJ, Bloemen LJ, Carson ML. Cancer risk for chemical workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Occup Environ Med 2003;60:672–5.
13. Consonni D, Pesatori AC, Zocchetti C, Sindaco R, cavalieri D'Oro L, Rubagotti M, et al. Mortality in a population exposed to dioxin after the

Seveso, Italy, accident in 1976: 25 years of follow-up. Am J Epidemiol 2008;167:847–58.

14. Neubert R, Maskow L, Triebig G, Broding HC, Jacob-Müller U, Helge H, et al. Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system: 3. Plasma immunoglobulins and cytokines of workers with quantified moderately-increased body burdens. Life Sci 2000;66:2123–42.

15. Kim HA, Ki EM, Park YC, Yu JY, Hong SK, Jeon SH, et al. Immunotoxicological effects of agent orange exposure to the Vietnam War Korean Veterans. Ind Health 2003;41:158–66.

16. Oh E, Lee E, Im H, Kang HS, Jung WW, Won NH, et al. Evaluation of immuno- and reproductive toxicities and association between immunotoxicological and genotoxicological parameters in waste incineration workers. Toxicology 2005;210:65–80.

17. Saberi Hosnijeh F, Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Plasma cytokine concentrations in workers exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). Front Oncol 2012;2:37.

18. Bueno de Mesquita HB, Doornbos G, van der Kuip DA, Kogevinas M, Winkelmann R. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. Am J Ind Med 1993;23:289–300.

19. Boers D, Portengen L, Turner WE, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Plasma dioxin levels and cause-specific mortality in an occupational cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. Occup Environ Med 2012;69:113–8.

20. Pellegrini P, Berghella AM, Contasta I, Adorno D. CD30 antigen: not a physiological marker for TH2 cells but an important costimulator molecule in the regulation of the balance between TH1/TH2 response. Transpl Immunol 2003;12:49–61.

21. Nolte MA, Van Olffen RW, Van Gisbergen KP, Van Lier RA. Timing and tuning of CD27–CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. Immunol Rev 2009;229:216–31.

22. Zinzani PL, Pileri S, Bendandi M, Buzzi M, Sabattini E, Ascani S, et al. Clinical implications of serum levels of soluble CD30 in 70 adult anaplastic large-cell lymphoma patients. J Clin Oncol 1998;16:1532–7.

23. Nilsson A, de Milito A, Mowafi F, Winberg G, Björk O, Wolpert EZ, et al. Expression of CD27–CD70 on early B cell progenitors in the bone marrow: implication for diagnosis and therapy of childhood ALL. Exp Hematol 2005;33:1500–7.

24. Mondria T, Lamers CH, Boekhorst PA, Gratama JW, Hintzen RQ. Bone-marrow transplantation fails to halt intrathecal lymphocyte activation in multiple sclerosis. J Neurol Neurosurg Psychiatry 2008;79:1013–5.

25. Grulich AE, Vajdic CM, Cozen W. Altered immunity as a risk factor for non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 2007;16:405–8.

26. Marshall NB, Kerkvliet NI. Dioxin and immune regulation: emerging role of aryl hydrocarbon receptor in the generation of regulatory T cells. Ann N Y Acad Sci 2010;1183:25–37.

27. Saberi Hosnijeh F, Heederik D, Vermeulen R. A review of the role of lymphoma markers and occupational and environmental exposures. Vet Q 2012;32:61–73.

28. Perrier S, Darakhshan F, Hajduch E. IL-1 receptor antagonist in metabolic diseases: Dr Jekyll or Mr Hyde? FEBS Lett 2006;580:6289–94.

29. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006;56:69–83.

30. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN∗2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998;47:195–8.

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