Immunological markers among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin

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Abstract

***Objectives*—To examine the association of immune cell number and function with occupational exposure to substances contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).**

***Methods*—A cross sectional medical survey. The exposed participants were employed at two chemical plants between 1951 and 1972 in the manufacture of 2,4,5-trichlorophenate and its derivatives. The reference group consisted of people with no occupational exposure to phenoxy herbicides who lived within the communities of the workers. Data from a total of 259 workers and 243 unexposed referents were included in the analysis of immune function. Laboratory tests for immune status included enumeration of circulating leukocyte and lymphocyte populations, proliferative responses of circulating lymphocytes to mitogens and antigens, and serum concentrations of the major immunoglobulins and complement factor C3.**

***Results*—The workers had substantial exposure to substances contaminated with TCDD, as indicated by a lipid adjusted mean serum TCDD concentration of 229 ppt compared with a mean of 6 ppt in the unexposed referents. Workers were divided into categories based on their serum TCDD concentration. For all categories except the lowest, with values of serum TCDD comparable with the unexposed referents, there were increased odds of having lower counts of CD26 cells (activated T cells) (odds ratio (OR) 1.0, 95% confidence interval (95% CI) 0.5 to 1.8 for TCDD <20 ppt; OR 1.6, 95% CI 0.8 to 3.2 for TCDD 20–51 ppt; OR 2.7, 95% CI 1.4 to 5.1 for TCDD 52–125 ppt; OR 2.6, 95% CI 1.4 to 4.9 for TCDD 125–297 ppt; OR 2.4, 95% CI 1.3 to 4.6 for TCDD 298–3389 ppt). A less consistent finding was decreased spontaneous proliferation of cultured lymphocytes. However, increases were found in proliferation of lymphocytes in response to concanavalin and pokeweed in workers in the high TCDD category. Age, cigarette smoking, and alcohol were significant predictors of several immunological outcomes.**

***Conclusions*—Associations between serum TCDD concentration and both a decrease in circulating CD26 cells and decreased spontaneous background proliferation were the major findings of this study. These results are unlikely to be of clinical importance but may reflect limited evidence for an association between immunological changes in workers and high serum concentrations of TCDD, or chance findings resulting from the evaluation of multiple immunological variables.**

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Experimental studies in laboratory animals have shown immunotoxicity associated with exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reflected by decrements in humoral and cell mediated immune responses, humoral antibody production and cell proliferation on antigen stimulation, and decreased complement function.[1–6] In inbred mice, the Ah receptor seems to play a major part.[1] By contrast, the few studies of adult human populations with substantial previous exposure to TCDD have provided limited and inconsistent evidence for immunotoxicity.[7–16] By contrast with the experimental studies, the humans usually have lower serum concentrations of TCDD, were exposed for much longer, and were usually adults when exposed. The human studies are limited by the size of the populations studied.

In 1986, Hoffman *et al*[7] studied 135 community residents potentially environmentally exposed to contaminated industrial sludge and reported a significantly decreased percentage of lymphocytes expressing CD3, CD4, and CD2, as well as increased prevalence of allergy to delayed skin test antigens. Although both findings are consistent with immune suppression, an increased pokeweed mitogen response suggestive of increased immunological responsiveness was also reported. This study did not include assessment of serum TCDD. In subsequent studies,[8 9] the findings of allergy were not replicated. However, by contrast with the original study, significant increases associated with higher concentrations of TCDD were found for IgG, %CD3, %CD8, number of T cells, percentages of CD2, and CD4/Leu-8 position.

Some of the highest levels of human exposure found have resulted from an industrial explosion and consequent community contamination in Seveso, Italy in 1976. In 1986, Sirchia *et al*[10 11] reported that 48 exposed children had higher titres of complement, increased lymphocyte responses to phytohaemagglutinin and pokeweed mitogen, and a tendency toward high numbers of peripheral blood lymphocytes.

In 1988, Jennings *et al*[12] investigated 18 workers exposed to TCDD 17 years previously in an industrial accident. Antinuclear antibodies, immune complexes, and the number of natural killer cells (monoclonal antibody leu-7) were significantly increased in the exposed workers. In 1991, Roegner *et al*[13] reported the results of studies conducted in 1987 of military personnel who were exposed to herbicides contaminated with TCDD during the Vietnam war. Significant increases were found in serum immunoglobulin A (IgA), but not IgG or IgM. Increases in cellular proliferation were found on stimulation by phytohaemmaglutinin. These findings, consistent with others previously cited, are opposite in direction to those expected if TCDD were associated with immune suppression.

In 1992 Zober *et al*[14] reported on 16 immunological variables for 42 workers exposed to brominated, rather than chlorinated dioxins or furans in resin production. An association was found with a decrease in total lymphocyte count, which was dependent on one person with the highest concentration of brominated dioxins.

In 1993, Neubert *et al*[15] reported on 89 workers involved in decontamination of a chemical plant where some had previously worked. The highest exposed category of 12 workers had a mean serum TCDD concentration of only 41.5 ppt. No significant decreases in immunological cell subsets were found with flow cytometry and multiple monoclonal antibody markers.

In 1994, Ott *et al*[16] reported on 138 workers exposed to TCDD in a 1953 trichlorophenol autoclave accident. Lower white blood counts in the exposed group were found but the extent of the decrease was inversely related to the TCDD concentration. Ott *et al* noted positive associations between TCDD and IgA and IgG concentrations, marginal increases in complement, and a marginal decline in lymphocytes.

To describe the possible association between exposure to TCDD and changes in the immune system, we studied a large group of United States workers with known substantial serum concentrations of TCDD, who had been exposed >15 years previously in the manufacture of herbicides and their precursors.

Methods

STUDY POPULATION

The study population of exposed workers was recruited from current and former employees of two of the 12 plants included in the National Institute for Occupational Safety and Health (NIOSH) cohort mortality study of workers exposed to TCDD.[17] At the two plants, TCDD exposure resulted from the manufacturing of 2,4,5-trichlorophenate (TCP) and its derivatives. Exposure to TCDD occurred between 1951 and 1969 at the New Jersey plant, and for about 2 years between 1968 and 1972 at the Missouri plant. Workers at both plants had potential exposure to other chemicals. We conducted a cross sectional medical study of workers from the two plants in 1987 and 1988.

A total of 586 workers, 490 from the New Jersey factory and 96 from Missouri were identified. Of the 586 workers, 143 were dead (24%), and 43 (7.3%) were not traced, leaving 400 workers (68%) eligible for participation in the study. A total of 357 of the eligible workers (89%) completed an interview, of whom 281 (70% of those eligible) also participated in the medical examination. The complete cohort has been described previously.[18] Eight of the exposed male workers were dropped from this analysis because they did not have a serum analysis for TCDD. Fourteen female workers were also excluded. One did not have a serum analysis for TCDD. Because only three of 13 exposed female workers had concentrations of serum TCDD higher than the referents, all female workers were excluded from the study. The remaining number in the exposed group was 259.

Two hundred and sixty current neighbourhood referents, with no previous employment in factories which produced phenoxy herbicides, participated in the medical examinations out of 325 referents who were interviewed. Age (within 5 years), race, and sex matched referents were identified by a door to door screening of each worker's census tract neighbourhood. Seventy five per cent of the referents represented the first, second, or third person identified as a potential candidate. Seventeen female referents were not included in the analysis. The remaining number in the referent group was 243.

Of the 260 potential referents, we analysed serum samples for TCDD from the first 20 who had medical examinations; all 20 had concentrations <20 ppt and a mean concentration of 8 ppt. Because assays were exceedingly expensive, we selected a random sample of the remaining referents for examination for TCDD resulting in serum concentrations for 79 referents. For epidemiological analysis, the referents not tested for serum TCDD were assigned the median serum TCDD concentration found in the tested referents, 6.08 ppt. Also, we estimated the concentration of serum TCDD for those whose serum concentrations fell below the limit of detection. The estimated concentration was calculated by dividing the limit of detection of each sample by the square root of two.[19]

Trained interviewers administered a questionnaire on occupational histories. Different interviewers, blinded to occupational histories, administered questionnaires on medical histories, and demographic and lifestyle characteristics, including smoking, alcohol consumption, education, and income. Medical examinations were conducted by physicians unaware of the exposure status of the participants.

LABORATORY METHODS

The collection, preparation, analytical technique, and quality control standards of the laboratory analyses for clinical chemistry and TCDD analysis have been presented.[18 20] A description of the laboratory methods for the analysis of the immune system, including a description of the quality control methods

Table 1 Lymphocyte subsets that were evaluated

Designation	*Description*	*Reagents*
CD3+	Mature T cells	Anti-T3
CD3+ CD26	Activated T cells	Anti-Ta1
CD4+	T helper-inducer cells	Anti-T4
CD4+ CD29+	T inducer of help	Anti-4B4
CD4+ CD45RA+	T inducer of suppression	Anti-2H4
CD8+	T cytotoxic-suppressor; some NK cells	Anti-T8
CD8+ CD11b+	T suppressor cells	Anti-CD11b
CD8+ CD11b-	T cytotoxic cells	Anti-CD11b
CD20	Mature B cells	Anti-B1
CD56	Most NK cells	Anti-NKH-1
CD4/CD8 ratio		
(CD4 + CD8)/CD3 ratio		

used, has been previously reported.[21] The immunological assays used to assess immune function included flow cytometric assessment of lymphocyte subsets, natural killer cell cytotoxic activity, and lymphocyte proliferative response after in vitro stimulation with mitogens and recall antigens.

Blood was collected in 10 U heparin/ml blood tubes. Peripheral blood mononuclear cells were obtained from a portion of the blood with standard Ficoll-Hypaque separation procedures. These peripheral blood mononuclear cells were used for lymphocyte subset analysis and the natural killer cell assay. The remaining whole blood was used in a whole blood lymphocyte stimulation assay. Peripheral blood mononuclear cells were analysed for lymphocyte subsets with fluorescence conjugated monoclonal antibodies. All reagents were obtained from Coulter Immunology, Hialeah, FL, USA. Non-specific IgG control antibodies were also used, and non-specific staining was subtracted from the percentage of positive cells. The data are presented as percentage of positive cells and absolute number of cells ($1000/mm^3$). The lymphocyte subsets evaluated are shown in table 1.

Natural killer cell activity was assessed with the ^{51}Cr release cytotoxicity assay. The K562 erythroleukaemia tumour cell line was used as the target cell, at effector:target ratios of 50:1, 25:1, 12:1, 6:1, and 3:1. The data are presented as lytic units (LU) with the method of Edwards *et al*,[22] with units of $LU/10^6$ effector cells. The lymphocyte proliferative response to mitogens and recall antigens was assessed with a whole blood method. The concentrations of mitogens used were: phytohaemagglutinin 0.25, 1.0, 4, 16, and 64 μg/ml, concanavalin A 1, 3, 9, 27, and 81 μg/ml, and pokeweed mitogen 18.8, 37.5, 75, 150, and 300 μg/ml. One dilution was used for each of the three antigens: mumps 1:100, *Candida* 1:20, and tetanus toxoid 1:100. At the end of the 7 day incubation, lymphocyte proliferation was assessed with ^3H-thymidine incorporation. The data are presented as the proliferative response (cpm) at the concentration of mitogen or antigen that resulted in the highest response, with the background proliferation subtracted out.

STATISTICAL ANALYSIS

As a first exploration of the data, we described the distributions of the immunological outcome variables with univariate statistics.[23] Then we categorised the exposed population into quintiles of TCDD (0–19 ppt, 20–51 ppt, 52–125 ppt, 126–297 ppt, and 298 to 3389 ppt). We included a category of exposed workers with concentrations comparable with the referents, <20 ppt, which is the maximum that has been found in people without occupational exposure.[20] We examined these six groups (five exposed; one non-exposed referent) for cohort membership, self reported smoking history (smoked within the past year), age at the time of examination, self reported alcohol consumption (drank alcohol within the past year), and serum TCDD.

Given the many tests for immune cell number and immune function, we sought to identify a subset of immunological outcomes for which we could use logistic regression analyses to assess their association with concentration of TCDD. Firstly, we compared all immunological outcomes between the referent population and the exposed population[23] and selected those with a significant difference (Students t test $p < 0.05$) for logistic analysis.

To augment the subset of immunological outcomes selected for later analysis with logistic regression, and to further explore potential relations between the many immunological variables assayed in this population and concentration of serum TCDD, we next used linear regression. For each immunological outcome variable, we forced serum TCDD into the analysis. We chose age, cigarette smoking, and alcohol consumption as potential predictors because of their general relation to health and disease. We added these potential predictors with a stepwise procedure. Potential predictors were retained in the final model if they increased the R^2 by $\geq 0.5\%$. To evaluate the possibility for a curvilinear relation between the immunological outcomes and TCDD, we entered a TCDD squared term into the final equation and inspected scattergrams. Immunological outcome variables that were significantly associated with TCDD in the regression analyses at a liberal p value of 0.1 were selected for analysis by logistic regression.

We chose logistic regression for its ease in modelling the association of the many immunological outcome variables with concentration of TCDD. For each logistic regression analysis, exposed workers and referents were categorised into one of six categories previously described. For a test for trend, serum TCDD was entered as a continuous variable and alternatively as a categorical variable. Age, cigarette smoking, and alcohol consumption were included as possible confounding variables. Interaction terms between TCDD and possible confounding variables were evaluated.

To establish case and referent definitions for immunological outcome variables for the logistic regression, we divided the referents into tertiles according to their values on each immunological outcome. Dependent on whether the relation in linear regression was positively or negatively associated with TCDD, or the mean for the outcome among the exposed population

Table 2 Demographic characteristics according to serum 2,3,7,8-TCDD category for chemical workers and referents from New Jersey and Missouri, 1987

Serum 2,3,7,8-TCCD category (pg/g)	*n*	*Age (mean (SD))*	*NJ cohort (%)*	*Current smokers (%)*	*Former smokers (%)*	*Current alcohol drinkers (%)*	*Former alcohol drinkers (%)*	*TCDD (mean (pg/g))*
Referents:								
0–19	243	56.1 (10.6)	77.0	33.2	45.2	62.6	25.1	6.4
Workers:								
0–19	65	52.4 (9.2)	76.9	40.6	39.1	57.4	31.1	10.8
20–51	47	54.1 (10.6)	78.7	43.5	32.6	56.5	39.1	32.5
52–125	49	53.2 (10.1)	67.4	35.4	41.7	73.4	20.4	84.3
126–297	49	54.4 (9.0)	73.5	24.5	53.1	70.8	25.0	201.0
298–3389	49	63.1 (10.0)	89.8	21.3	55.3	61.2	26.5	880.5

was above or below that of the reference population, we then used the cut off values for the highest tertile of the referents (if the association was positive) or lowest tertile (if the association was negative) to define the boundary between cases and controls for logistic regression. This forced the prevalence of cases in the reference population to be about 1 in 3.

Results

This study population consisted of 259 exposed workers and 243 local referents. The age of the study population ranged from 31 to 77. The distribution of age, cohort, smoking, drinking, and serum TCDD, by category of serum TCDD are presented in table 2. The age distribution, alcohol history, and cohort mem-

Table 3 Comparison of immunological outcomes for chemical workers and referents from New Jersey and Missouri, 1987

	Exposed (n=259)		*Referents (n=243)*		
Variable	*Mean*	*SD*	*Mean*	*SD*	*p Value*
Morphology:					
White blood cells (k/mm^3)	6.9	2.0	7.0	2.6	0.57
Segmented neutrophils (%)	52.8	8.3	51.4	9.0	0.08
Band neutrophils (%)	5.4	3.7	6.2	4.2	0.02
Mononuclear cells (%)	7.5	2.5	7.2	2.9	0.30
Eosinophils (%)	2.4	1.9	2.3	1.9	0.53
Basophhils (%)	0.4	0.5	0.5	0.5	0.59
Lymphocytes (%)	29.4	8.5	30.3	9.6	0.29
Atyypical lympocytes (%)	2.1	3.0	2.1	2.4	0.81
Immune markers:					
CD20*	0.2	0.1	0.2	0.1	0.56
CD20†	9.8	4.2	9.5	4.2	0.57
CD3 (%)	72.1	9.3	72.9	10.0	0.35
CD3 (k/mm^3)	1.5	0.5	1.6	0.2	0.19
CD3/Ta1 (k/mm^3)	0.02	0.02	0.02	0.03	0.0007
CD3/Ta1 (%)	0.7	1.2	1.1	1.5	0.0015
CD4 (k/mm^3)	1.1	0.4	1.1	0.4	0.50
CD4 (%)	49.9	9.6	49.9	10.3	0.96
CD4/CDw29 (k/mm^3)	0.6	0.3	0.7	0.3	0.06
CD4/CDw29 (%)	29.2	8.5	30.7	9.1	0.05
CD4/CD45 (k/mm^3)	0.4	0.3	0.4	0.3	0.16
CD4/CD45 (%)	18.5	9.3	19.4	9.7	0.32
CD8 (k/mm^3)	0.6	0.3	0.6	0.3	0.30
CD8 (%)	27.5	8.8	27.7	9.5	0.75
CD8/CD11b+ (k/mm^3)	0.1	0.1	0.1	0.1	0.28
CD8/CD11b+ (%)	4.3	3.4	4.6	3.4	0.23
CD8/CD11b− (k/mm^3)	0.5	0.3	0.5	0.3	0.40
CD8/CD11b− (%)	23.2	8.6	23.2	9.7	0.97
CD56 (k/mm^3)	0.4	0.2	0.4	0.2	0.54
CD56 (%)	19.2	8.7	18.2	7.7	0.15
Ratios:					
CD4/CD8	2.1	1.1	2.1	1.1	0.80
(CD4 + CD8)/CD3	1.1	0.1	1.1	0.1	0.21
Immunoglobulins:					
IGA (mg/dl)	255.7	120.3	262.6	128.0	0.53
IGG (mg/dl)	1126.4	278.5	1216.2	392.0	0.003
IGM (mg/dl)	128.1	81.6	127.5	60.9	0.93
Complement mg/dl	90.5	21.3	86.1	21.0	0.02
Stimulation tests:					
Background proliferation‡ (mitogen assay)	569.0	1062.2	678.6	564.7	0.15
Background proliferation‡ (antigen assay)	441.8	279.3	563.9	404.7	0.0001
Concanavilin§	92733.1	35796.1	93754.1	63751.4	0.83
Phytohemaglutinin§	134016.0	55111.0	130843.5	48400.2	0.50
Pokewee§	29581.2	13536.3	27852.7	14080.1	0.17
Tetanus antigen§	6910.7	14173.1	7708.7	13594.1	0.53
Candida antigen§	9517.5	16090.9	7699.6	13142.8	0.17
Mumps antigen§	1190.2	4575.3	1209.5	3568.1	0.96
Natural killer cells					
Lytic units	176.9	150.7	134.7	112.3	0.0006

*Absolute values are calculated as follows—for example, for B lymphocytes=CD20 absolute=(lymphocytes+atypical lympocytes)/100×white blood cells×(CD20/relative)/100.
†Relative results are read from the flow cytometer and are reported as %.
‡Proliferation without added antigen or mitogen.
§Highest proliferation regardless of dilution minus background.

Table 4 Logistic regression by serum 2,3,7,8-TCDD category for chemical workers and referents from New Jersey and Missouri, 1987

Variable	*Case definition*	*TCDD 0–19 OR (95% CI)*	*TCDD 20–51 OR (95% CI)*	*TCDD 52–125 OR (95% CI)*	*TCDD 126–297 OR (95% CI)*	*TCDD 298–3389 OR (95% CI)*
Morphology:						
Segmented neutrophils (%)	GE 56	0.7 (0.4 to 1.3)	1.3 (0.7 to 2.5)	1.4 (0.7 to 2.7)	1.5 (0.8 to 2.8)	1.0 (0.5 to 1.9)
Banded neutrophils (%)	LT 4	1.6 (0.9 to 2.9)	3.0 (1.6 to 5.8)	1.7 (0.9 to 3.3)	1.3 (0.7 to 2.6)	2.0 (1.1 to 3.9)
Lymphocytes (%)	LT 26	0.8 (0.4 to 1.5)	0.9 (0.4 to 1.9)	1.6 (0.8 to 3.2)	2.9 (1.5 to 5.5)	0.8 (0.4 to 1.7)
Immune markers:						
CD3/Ta1 (k/mm^3)	LT0.004	1.0 (0.5 to 1.8)	1.6 (0.8 to 3.2)	2.7 (1.4 to 5.1)	2.6 (1.4 to 4.9)	2.4 (1.3 to 4.6)
CD3/Ta1 (%)	LT 0.24	1.2 (0.7 to 2.2)	1.5 (0.8 to 2.9)	2.5 (1.3 to 4.8)	2.3 (1.2 to 4.3)	2.4 (1.3 to 4.5)
CD4/CDW29 (k/mm^3)	LT 0.52	1.3 (0.7 to 2.4)	1.3 (0.7 to 2.7)	1.3 (0.6 to 2.5)	1.5 (0.8 to 2.8)	1.3 (0.7 to 2.6)
CD4/CDW29 (%)	LT 26.83	1.3 (0.7 to 2.3)	1.1 (0.6 to 2.2)	1.6 (0.8 to 3.0)	1.0 (0.5 to 1.9)	1.6 (0.8 to 3.0)
CD4/CD45 (k/mm^3)	LT 0.27	1.6 (0.9 to 2.9)	1.0 (0.5 to 2.0)	0.9 (0.4 to 1.7)	1.2 (0.6 to 2.3)	1.7 (0.9 to 3.3)
CD4/CD45 (%)	LT 14.68	1.6 (0.9 to 2.8)	1.2 (0.6 to 2.3)	1.1 (0.5 to 2.1)	0.6 (0.3 to 1.2)	1.4 (0.7 to 2.6)
CD8/CD11B+ (k/mm^3)	LT 0.055	0.9 (0.5 to 1.7)	1.0 (0.5 to 2.0)	1.1 (0.6 to 2.2)	1.3 (0.7 to 2.4)	2.3 (1.2 to 4.4)
CD8/CD11B+ (%)	LT 10.5	0.4 (0.2 to 1.1)	0.8 (0.2 to 3.1)	2.7 (0.3 to 21.2)	1.4 (0.3 to 6.6)	1.8 (0.4 to 8.4)
CD8/CD11B− (k/mm^3)	GE 0.539	1.5 (0.9 to 2.7)	0.5 (0.2 to 1.1)	1.2 (0.6 to 2.3)	0.9 (0.4 to 1.8)	1.4 (0.7 to 2.7)
Immunoglobulins:						
IgG	LT 1015	2.5 (1.4 to 4.4)	1.5 (0.7 to 3.0)	1.9 (1.0 to 3.6)	1.5 (0.8 to 3.0)	2.1 (1.1 to 4.1)
Complement (mg/dl)	GE 94	1.0 (0.6 to 1.9)	2.3 (1.2 to 4.5)	1.0 (0.5 to 1.9)	1.0 (0.5 to 2.0)	1.0 (0.5 to 2.0)
Stimulation to tests						
Concanavilin	GE 99337	0.7 (0.4 to 1.4)	1.3 (0.7 to 2.6)	1.0 (0.5 to 2.0)	1.3 (0.7 to 2.5)	3.2 (1.7 to 6.2)
Phytohaemaglutinin	GE 149423	0.7 (0.4 to 1.3)	1.3 (0.7 to 2.5)	1.0 (0.5 to 1.8)	1.0 (0.5 to 1.9)	1.5 (0.8 to 2.9)
Pokeweed	GE 30145	0.7 (0.4 to 1.3)	1.2 (0.6 to 2.4)	1.2 (0.6 to 2.2)	1.6 (0.8 to 3.0)	1.8 (1.0 to 3.5)
Background proliferation (mitogen assay)	GE 613	0.6 (0.3 to 1.0)	0.4 (0.2 to 0.8)	0.4 (0.2 to 0.8)	0.5 (0.2 to 0.9)	0.4 (0.2 to 0.7)
Background proliferation (antigen assay)	LE 334	1.1 (0.6 to 1.9)	1.0 (0.5 to 2.0)	1.9 (1.0 to 3.6)	1.5 (0.8 to 2.9)	2.2 (1.2 to 4.2)
Natural killer cell						
Lytic units	GE 140.8	2.2 (1.2 to 4.0	1.3 (0.7 to 2.6)	1.9 (1.0 to 3.7)	1.7 (0.9 to 3.1)	1.7 (0.9 to 3.2)

bership (NJ *v* Missouri) were similar across strata of serum TCDD. There was an inverse relation between smoking and stratum of TCDD among the workers. Workers in the four highest strata of TCDD had mean TCDD concentrations that were substantially higher than referents and other unexposed populations living in industrialised countries that have been examined.[20]

Table 3 compares the means for the immune outcomes between the workers and referents. When compared with the referents, significant decreases were found for neutrophil bands, CD26, CD4/CD29, IgG, and background proliferation. Significant increases were found for complement and natural killer cell activity measured in lytic units.

In linear regression, two R^2 values were calculated; one in which TCDD was the only predictor in the model, and a second in which other predictors were included in the full model. The full models included TCDD and other variables that added at least 0.5% to the R^2 value for the model. The β coefficients for TCDD (adjusted for age, smoking, and alcohol as appropriate) were significantly negatively associated ($p<0.1$) for lymphocyte %, CD26 absolute number and %, CD45RA+ absolute number and %, CD8/CD11b+ absolute number and %, serum IgG, and background proliferation in the antigen assay. Significantly positive associations were found for segmented polymorphs %, CD8/CD11B- absolute count, background proliferation in the mitogen assay, and proliferation response to concanavalin, phytohaemaglutinin, and pokeweed. However the explanatory contribution of TCDD to any of these immunological outcomes was not substantial, maximally explaining 1%–2% of the variation of the immunological outcomes. The full models including either age, smoking, and alcohol explained variation of the immune outcomes ranging up to 12% for white blood cells. Inspection of scattergrams did not show relations between TCDD and the immunological outcome variables that were not linear.

Table 4 presents results of a logistic regression in which five concentrations of serum TCDD were compared with the unexposed referents, older age with younger age, former smokers with never smokers, current smokers with never smokers, former drinkers with never drinkers, and current drinkers with never drinkers. Age and current smoking, and to a lesser extent, current drinking and former smoking were associated with several immunological outcomes after adjustment for the other variables in the model.

There were also associations between categorised level of TCDD by strata and several immunological outcomes. For only one outcome, CD26 (activated T cells) both as lymphocytes % and as absolute count, was there a consistent association in all but the lowest two strata of exposed workers. For CD26, the relation with serum TCDD was inverse. Strata with higher concentrations of TCDD showed increased odds of having decreased absolute counts and proportions of CD26 cells. The effect seemed to plateau rather than to reflect a dose response relation.

A consistent pattern of decreased background proliferation is evident in the mitogen assays from exposed participants, and less evident in the antigen assay. For several variables including neutrophil bands, CD8/CD11B+ as absolute count, and proliferation response to concanavalin and pokeweed, there are significant excesses only in the highest category of TCDD. For other variables, there are excesses for individual TCDD categories, but not a consistent relation across TCDD categories.

As we assigned a serum dioxin concentration of 6.08 ppt to 174 referents who did not have their serum assayed for TCDD, we also conducted parallel logistic regression analyses eliminating referents with assigned values. The patterns of odds ratios for strata of TCDD and confounders were similar to the results including all referents, although fewer were significant. Controlling for the date of laboratory examination did not substantively alter the

Table 4 continued

χ^2test for trend p value	>−55/<55 age OR (95% CI)	Former smoker OR (95% CI)	Current smoker OR (95% CI)	Former drinker OR (95% CI)	Current drinker OR (95% CI)
0.17	2.5 (1.7 to 3.8)	0.6 (0.4 to 1.0)	1.1 (0.6 to 1.8)	1.0 (0.5 to 2.0)	0.7 (0.4 to 1.3)
0.52	0.7 (0.5 to 1.1)	1.2 (0.7 to 1.9)	0.9 (0.5 to 1.5)	0.6 (0.3 to 1.2)	0.7 (0.4 to 1.3)
0.15	2.4 (1.6 to 3.7)	0.6 (0.4 to 1.1)	1.2 (0.7 to 2.1)	1.5 (0.7 to 3.2)	1.4 (0.7 to 2.8)
0.01	1.7 (1.2 to 2.6)	0.9 (0.5 to 1.5)	0.8 (0.5 to 1.4)	0.7 (0.3 to 1.3)	0.8 (0.4 to 1.5)
0.03	1.6 (1.1 to 2.4)	0.8 (0.5 to 1.4)	0.9 (0.5 to 1.5)	0.8 (0.4 to 1.6)	0.9 (0.5 to 1.6)
0.70	1.2 (0.8 to 1.8)	0.8 (0.5 to 1.2)	0.3 ((0.2 to 0.5)	0.6 (0.3 to 1.2)	0.8 (0.5 to 1.6)
0.94	1.1 (0.7 to 1.6)	0.9 (0.6 to 1.5)	0.6 (0.3 to 0.9)	0.8 (0.4 to 1.5)	0.8 (0.4 to 1.5)
0.94	0.9 (0.6 to 1.3)	1.1 (0.6 to 1.7)	0.9 (0.5 to 1.5)	0.5 (0.2 to 0.9)	0.6 (0.3 to 1.1)
0.37	1.3 (0.9 to 1.9)	0.9 (0.6 to 1.5)	1.2 (0.7 to 1.9)	0.8 (0.4 to 1.5)	0.9 (0.5 to 1.7)
0.02	1.4 (0.9 to 2.0)	0.9 (0.6 to 1.6)	1.4 (0.8 to 2.4)	1.9 (0.9 to 4.0)	2.2 (1.1 to 4.5)
0.07	0.5 (0.2 to 1.2)	2.7 (1.1 to 6.8)	3.2 (1.1 to 8.9)	2.8 (0.7 to 10.9)	1.3 (0.5 to 3.6)
0.78	0.7 (0.4 to 1.0)	0.9 (0.5 to 1.5)	1.6 (1.0 to 2.7)	0.9 (0.5 to 1.8)	0.9 (0.5 to 1.6)
0.71	1.5 (1.0 to 2.2)	1.6 (0.9 to 2.8)	2.5 (1.5 to 4.5)	0.5 (0.2 to 1.0)	0.9 (0.5 to 1.7)
0.45	1.2 (0.8 to 1.8)	0.8 (0.5 to 1.3)	0.8 (0.4 to 1.3)	0.8 (0.5 to 1.7)	0.6 (0.3 to 1.1)
0.01	0.4 (0.3 to 0.7)	1.1 (0.6 to 1.8)	1.6 (0.9 to 2.7)	1.0 (0.5 to 2.2)	1.9 (1.0 to 3.8)
0.19	0.8 (0.5 to 1.2)	1.3 (0.8 to 2.2)	1.2 (0.7 to 2.0)	1.1 (0.5 to 2.4)	1.7 (0.9 to 3.4)
0.02	1.0 (0.7 to 1.5)	1.6 (0.9 to 2.7)	2.7 (1.5 to 4.6)	1.3 (0.6 to 2.7)	1.6 (0.8 to 3.2)
0.35	1.0 (0.7 to 1.5)	1.1 (0.7 to 1.8)	1.0 (0.6 to 1.6)	1.3 (0.7 to 2.6)	0.9 (0.5 to 1.6)
0.07	1.1 (0.7 to 1.6)	1.1 (0.7 to 1.8)	1.0 (0.6 to 1.7)	0.9 (0.4 to 1.8)	1.2 (0.6 to 2.2)
0.99	1.3 (0.9 to 2.0)	0.9 (0.6 to 1.5)	0.6 (0.4 to 1.1)	1.0 (0.5 to 2.0)	1.4 (0.8 to 2.7)

results. Interaction terms were not significant predictors.

Discussion

This study of chemical workers with substantial and long standing serum concentrations of TCDD considered many potential associations with immunological outcomes. Comparison of means for these outcomes and linear regression were used to identify a smaller number of outcomes that were modelled in relation to strata of TCDD among exposed and referent workers with logistic regression.

Of the tests that enumerated peripheral blood leucocytes and lymphocyte subsets, only two populations showed evidence of a possible association with TCDD exposure: the percentage of bands and the percentage and total number of activated T cells. Banded neutrophils were assessed from Wright stained blood smears by an automated differential counter, which generally provides more precise classification than manual methods.[24] However, the magnitude of the effect associated with TCDD was extremely small compared with normal biological variation: the difference between means was only about 20% of the standard deviation. Moreover, the relation with TCDD concentration, although increased in all strata of TCDD, only reached significance in two strata. This finding may well be due to chance and in any event is unlikely to be of clinical significance.

The apparent association between TCDD and a lower number of activated T cells in peripheral blood is more interesting for several reasons. Activated lymphocytes are quickly removed from circulation and therefore normally comprise only a small proportion of peripheral blood lymphocytes. The TA1 marker is now known to be a part (epitope) of the cell surface peptidase designated CD26. Although CD26 is present on both resting and activated T cells, the TA1 epitope is much more prominent on cells that have entered a growth cycle after an antigenic or mitogenic activation signal.[25] The CD26 phenotype is thus strongly correlated with T cell activation and proliferation.[26] Our results found that the exposed cohort had a significant decrease in both the proportion and total number of circulating activated T cells. Logistic regression analysis also showed a consistent dose-response relation: the odds ratio of a decrease in either percentage or total number showed no effect at the lowest TCDD category, increased across the 20–51 and 52–125 ppt strata, and plateaued at the highest stratum.

Finally, a decrease in activated T cells may be consistent with an incidental finding from the proliferation studies. Lymphocyte proliferative assays performed by incubating blood cells with stimulants (mitogens or antigens) included a control to measure background activity in the absence of stimulation. Although much of the background activity is purely noise, some contribution may be from spontaneous proliferation by the few preactivated lymphocytes harvested from the blood. The data from these assays show a small but significant difference in background activity in the antigen driven proliferative assay between the exposed and referent cohorts, such that the mean background activity measurement is lower in the exposed cohort. A comparable but less significant difference is present in the background activity in the mitogen driven assay, in which incubation lasted only 5 days, compared with 7 days for the antigen driven assay. Along with the lower numbers of CD26 cells in the exposed cohort, the data suggest that lymphocytes harvested from the peripheral blood of exposed workers had slightly lower numbers of activated T cells. The squared Pearson correlation coefficient for number of CD26 cells and background proliferation in the antigen test is 0.11 and 0.05 in the mitogen test. Although it explains only about 5%–10% of the variation, the direction of this correlation supports the possibility of a relation between the number of preactivated T cells and the degree of unstimulated proliferation. Other

sources of variation, including the imprecision of the assay, apparently contribute to the variance. Although the small size of the differences mitigates against any physiological relevance, it may reflect a subtle immune perturbation.

No association was found with CD4 helper or CD8 suppressor cells or their ratios. Assessment of humoral immunity was limited to analysis of CD20 cells—that is, B cells and immunoglobulins. We found no association of TCDD with CD20 cells. Among the immunoglobulins there seems to be a decrease in serum IgG concentrations, albeit not a significant finding in all categories of TCDD. Several of the earlier human studies reported increases in immunoglobulin concentrations, rather than decreases. There seems to be a tendency toward decreased spontaneous proliferation in the lymphocytes harvested from exposed workers as compared with lymphocytes harvested from the referents. Decreased spontaneous proliferation in vitro suggests some permanent effect of TCDD that persists when the cells are grown in vitro, removed from their exposed hosts. Analysis of cell proliferation on stimulation provides limited evidence for a positive association of TCDD with concanavalin and pokeweed, but not phytohaemagglutinin, mumps, or tetanus. Stimulation tests provide evidence for the competency of lymphocytes to recognise and respond to common antigens.

There has been limited study of workers or community residents with substantial exposure to TCDD or evidence of increased serum TCDD long after the end of exposure with which to contrast our results. Similar to the study of Hoffman *et al*[7] of community residents exposed to soil contaminated with TCDD, this study also provides limited evidence for an association with increased proliferation on stimulation with pokeweed mitogen and concanavalin that is not suggestive of immune suppression. This study does not lend support to the findings of Hoffman *et al*,[7] Zober *et al*,[14] and Ott *et al*[16]of decreased T cell subsets, except for the finding of decreased CD26 (activated T cells). Consistent with the studies of Evan *et al*[9]) and Ott *et al*[16], we found weak evidence for diminished concentrations of IgG but no association with IgA. Unlike the study of Roegner *et al*[13] of Air Force veterans, we did not find evidence of an increase in serum IgA. Unlike the study of Jennings *et al*[12] in exposed industrial workers, we do not provide evidence for an association with increased natural killer cells, albeit our data do provide limited evidence for increased lytic activity. By contrast with the study of Mocarelli *et al*[11] in children exposed at Seveso and the study of Ott *et al*[16] in industrial workers, we did not find evidence of increased complement. Our study does seem fairly consistent with these other studies in not providing substantial evidence for an association of TCDD with decreases in cell mediated immunity, humoral immunity, or natural killer cell activity. Our results specifically and the body of literature in general seem inconsistent with the literature on experimental animals.

There are several possible explanations for our findings, especially those that are positive. Firstly, we examined many immunological outcomes which is an approach comparable with panels of immunological tests used in experimental laboratory studies. Multiple comparisons should temper the interpretation of statistical test results. Chance, although a likely explanation for a random positive result when many associations are examined, is less likely to explain a finding that is consistent across increasing concentrations of TCDD strata, as we found with activated T cells. Isolated associations, when limited to the highest TCDD strata, may be the result of an effect limited to the highest level of exposure, essentially a threshold effect, a weak trend for which the effect is only large enough to be detected in the highest exposure level, or may represent an isolated chance finding found in the highest exposure stratum. It is possible, although unlikely, that this cross sectional study of survivors failed to detect a more pronounced immunological phenomenon among those who did not participate in this study or died previously, the so called survivor bias.

There may be a more substantive explanation for our lack of positive associations, especially when viewed contrasting with the profound effects of TCDD on experimental animals. One reassuring explanation is dose. The participants in this study have among the highest concentrations of serum TCDD that have been found in occupational cohorts, and certainly higher than have been found in environmental exposures with the exception of Seveso. It may be that TCDD does have an immunotoxic effect in humans, albeit an effect that is not discernable at the levels of exposure so far found in humans. Alternatively, the lack of an obvious immunotoxic effect in humans may reflect the inherent importance of the AH receptor in manifesting immunotoxicity.[1] We have previously studied[27] *P*-450 induction by TCDD in this same population with ratios of urinary caffeine metabolites as an experimental measure of *P*-450—that is, human AH enzyme induction. We found little evidence for inducibility. If immunotoxicity is related to inducibility of human AH, and assuming the validity of urinary caffeine metabolites as a marker of *P*-450 activity, then the lack of evidence for inducibility in humans at these levels of exposure to TCDD may explain the lack of immunotoxicity.

The most interesting hypothesis to emerge from our results is that TCDD is associated with a decrease in the background concentration of T cell activation, as manifest by the decreased number and proportion of CD26 cells and supported by the decrease in spontaneous proliferation of lymphocytes harvested from exposed workers. If this is true, the lowering of background activity is readily overcome in vitro by stimulation with either antigens or mitogens and thus is unlikely to have any clinical importance in the participants' ability to ward off infection.

We think that the primary value of this study is in evaluation of possible associations, or lack

thereof, between immunological outcomes and serum TCDD in a large population of substantially exposed industrial workers many years after exposure. These data provide a basis to speculate upon the associations that we found, but no evidence that TCDD caused meaningful immunological changes in this exposed cohort.

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