Immunologic Findings in Workers Formerly Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and its Congeners

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One hundred ninety-two workers in a German pesticide factory who were exposed to polychlorinated dibenzodioxins and -furans (PCDD/PCDF) were investigated for former and present diseases and laboratory changes of the immune system. Moreover, in a subgroup of 29 highly exposed and 28 control persons, proliferation studies were performed. In addition to assays such as blood count, immunoglobulins, serum electrophoresis, monoclonal bands, surface markers, autoantibodies, and lymphocyte proliferation, two new methods, the rise of tetanus antibody concentration after vaccination and the *in vitro* resistance of lymphocytes to chromate, were used to diagnose the morphologic and functional state of the immune system. There was no stringent correlation of actual PCDD/PCDF concentrations with the occurrence of infections or with one of the immune parameters. In addition, outcomes of the tetanus vaccination and the chromate resistance test were not correlated with PCDD/PCDF. However, the chromate resistance of lymphocytes stimulated by phytohemagglutinin of highly exposed persons was significantly lower than that for the control group. These findings indicate that the function of lymphocytes can be stressed and possibly impaired by high exposure to PCDD/PCDF. — *Environ Health Perspect* 106(Suppl 2):689–695 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/689-695jung/abstract.html*

Key words: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD, immunology, human, epidemiology, proliferation, surface markers, autoantibodies, vaccination

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and its congeners are well known immunotoxicants. Previous studies point to the B cell as the primary target of the immunotoxicity of TCDD (*1–3*). B cells are a very sensitive target of TCDD even in humans; concentrations of the toxin *in vitro* as low as 10^{-14} mol were capable of reducing the number of these cells (*4*). However, these findings could not be corroborated in a later study (*5*). The development of T cells in animals is also impaired by TCDD because of damage to the thymic epithelium and the lymphoid precursor cells before they enter the thymus (*6–8*). Reports about the damage of other immunocompetent cells (natural killer cells, cytotoxic T lymphocytes, polymorphonuclear cells) are equivocal (*9–14*).

Existing epidemiologic studies in humans have not revealed consistent patterns of damage to the immune system by TCDD and its congeners. To date, except for one morbidity study (*15*) in which an increased incidence of infectious diseases, especially appendicitis, was found in TCDD-exposed individuals, all epidemiologic studies have been restricted to evaluation of laboratory parameters.

The number of circulating lymphocytes in exposed persons was found to be lower immediately after but not a year after the accident in Seveso, Italy (*16*). After environmental exposure they were lowered in persons living in Quail Run, Missouri (*17*), but increased in persons in Times Beach, Missouri (*18*). The number of circulating lymphocytes was elevated among inhabitants of Vietnam exposed to Agent Orange and therefore TCDD (*19*).

There was no change in the lymphocyte subgroups in members of the exposed population of Seveso (*16*) and in decontamination workers (*20*). A decrease of $CD4^+$ cells and of the T4/T8 ratio was seen in the cohort of Quail Run (*17,21*), a rise of $CD8^{\pm}$ cells in the Times Beach cohort (*18*), and a decrease of these cells in the Badische Anilin- und Sodafabrik cohort (*22*). Natural killer cells were slightly lowered (*22*) or increased (*23*) after occupational exposure.

The function of lymphocytes was impaired in a cohort formerly working in a plant where 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was produced (*24*) and in the initial investigation of the Quail Run cohort (*17*). Later investigations in the latter cohort did not corroborate these findings (*21*). In the Seveso and Times Beach cohorts and in decontamination workers proliferation of lymphocytes of exposed persons was undisturbed (*16,18,25*). A lowered ability of phagocytosis of the blood cells was reported among exposed Vietnamese subjects (*19*).

This paper is based on a presentation at the International Symposium on Dioxins and Furans: Epidemiologic Assessment of Cancer Risks and Other Human Health Effects held 7–8 November 1996 in Heidelberg, Germany. Manuscript received at *EHP* 28 May 1997; accepted 14 November 1997.

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Tetanus toxoid was a kind gift of Behring, Marburg, Germany. The analyses of PCDD/PCDF in 135 blood specimens were funded by the National Institute of Environmental Health Sciences through an interagency agreement with the Centers for Disease Control, contract Y1-ES-0072. Proliferation studies are from the M.D. thesis of C. Koitka (University of Mainz, Mainz, Germany). Informed consent was obtained from all involved human study subjects.

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Abbreviations used: ESR, erythrocyte sedimentation rate; PCDD/PCDF, polychlorinated dibenzo-*p*-dioxins/furans; PHA, phytohemagglutinin; PWM, pokeweed mitogen; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, TCDD equivalent dose; TET, tetanus toxoid.

The Multi-Merieux test (*17*), a test on the function of reagibility on delayed-type antigens, was not changed in the Boehringer cohort (*26*) and was depressed in the Quail Run cohort in 1986 (*17*) but not in 1989 (*21*).

Immunoglobulin (Ig)G concentration in blood was decreased (*26*), unchanged (*23*), and increased (*18,22*) after exposure to TCDD and its congeners. Components of the complement system were investigated in only two studies (*16,22*); in both studies, C3 and C4 concentrations in blood were increased. In two other studies, one on workers of a pesticide-producing factory (*23*) and the other on inhabitants of Vietnam (*19*), the concentrations of immune complexes were evaluated, in the Jennings et al. (*23*) study, the concentration of antinuclear antibodies in blood were raised.

So, some immune parameters in humans such as cell count, distribution of lymphocyte subsets, and the amount of immunoglobulins in serum are modulated by TCDD exposure in either direction; the following results are more consistent. The complement fraction is increased [though lowered in animal experiments (*27*)] under the influence of TCDD; immune complexes and autoantibodies in blood also increase with exposure. Human lymphocyte proliferation studies showed either no difference between the exposed and control groups or proliferation ability was lowered in accordance to exposure with polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzo-*p*-furans (PCDD/PCDF). In these human studies, therefore, impairment of lymphocyte function was more evident than morphological changes.

In this present study we examined a group of former workers of a pesticide-producing plant, who still exhibited high body burdens of TCDD and its congeners, for changes of morphologic and functional properties of the immune system.

Methods

Study Groups

Because of a contract after the closing of a German pesticide-producing factory in 1984, 450 former workers were offered comprehensive health status checks. One hundred ninety-two of these former workers participated in this investigation, which took place in Mainz and Hamburg, Germany, from 1992 to 1994. No information could be gained about why some workers refused to participate in the study.

Eight of the 192 persons participating in the study were women. Age of the study population ranged from 27 to 83 years of age, with a median age of 56. The range of TCDD concentrations was 1.2 to 893.2 pg/g fat with a median of 36.1 pg/g fat and a 95 percentile of 343.2 pg/g fat. The range of TCDD equivalent dose (TEQ) concentrations was 11.1 to 1153.1 pg/g fat with a median of 103.7 pg/g fat and a 95 percentile of 592.3 pg/g fat.

A subgroup of the 29 most highly exposed persons (S_H) was selected from the total cohort of 192 workers based on their work histories and the analyses of Flesch-Janys et al. (*28*). They were compared to a control group (S_C) of 28 external persons (gas workers) not exposed to PCDD/PCDF above the environmental level, as verified in a random sample of four persons. These groups were comparable in age, social status, and smoking habits (12 persons in the S_H group and 11 in the S_C group were current smokers). Median age of the exposed group S_H was 55.5 (range 37–69.3) years. The median TCDD concentration of the S_H group was 217 (range 33.6–2252) pg/g blood lipid and the median TEQ was 425.9 (range 73.9–2732.9) pg/g blood lipid. Twenty-one persons in the S_H group were production workers for more than 7.5 years in two departments of the former factory (the trichlorophenol and 2,4,5-T departments), with known high exposure to TCDD. The remaining eight persons were mechanics working in these departments. Median age of the unexposed group S_C was 53.8 (range 42.8–65.3) years, median TCDD concentration was 3.9 (range 2.9–6) pg/g blood lipid, and S_C median TEQ concentration was 15.4 (range 12–22.8) pg/g blood lipid.

Immunologic Studies

Study participants were asked on a questionnaire about their medical and work histories. Also, their health status was investigated clinically. They were asked especially about occurrence and frequency of infectious diseases, cancer, and previous or current drug intake. Measurements of immunologic parameters were obtained through blood and urine samples (Table 1).

Analysis of erythrocyte sedimentation rate (ESR), full blood count, and serum electrophoresis IgA, IgG, and IgM were determined with the Behring Nephelometer II Analyzer (Behring Diagnostics, Marburg, Germany) using the original fixed-time method and the original reagent kits of the Behring company.

Table 1. Number of participants in the respective investigations.

Investigation	Participants, no.
ESR	149
Full blood count	188
Serum electrophoresis	185
IgA, IgG, IgM	190
Specific and natural autoantibodies	149
Monoclonal antibodies in serum	149
L-Chains in urine	149
Lymphocyte surface markers	188
Proliferation of lymphocytes under the influence of PHA, PWM, tetanus toxoid	171
Tetanus antibodies before and 3 weeks after vaccination	53

Abbreviations: PHA, phytohemagglutin; PWM, pokeweed mitogen.

Specific autoantibodies in serum (against nuclei [ANA], smooth muscle, mitochondria, sarcolemma, vessel endothelium, liver sinusoids, heart muscle, parietal cells, thyroid gland, islet cells, and adrenal gland) were determined semiquantitatively by immunofluorescence. They were classified 0, negative; 1, weakly positive; 2, positive; 3, strongly positive, or 4, very strongly positive. A sum score over all different autoantibodies was formed by adding these scores.

Natural autoantibodies against microsomes, laminin, and keratin were determined by the enzyme-linked immunosorbent assay (ELISA) technique. Monoclonal antibodies and L-chains in urine were detected by an immune fixation test (Beckman, Munich, Germany).

Lymphocyte surface markers were measured by flow cytometry using the EPICS XLPE II, Coulter Electronics (Hamburg, Germany). Mononuclear cells were cryopreserved after controlled freezing and stained with monoclonal antibodies against CD3, CD4, CD8, CD16, CD19, CD23, CD25, CD56, and MHC class II to detect different lymphocyte subsets. All monoclonal antibodies were purchased by Coulter Immunotech and Diagnostics (Hamburg, Germany). The percentages of different lymphocyte subgroups and their absolute numbers were obtained by double staining.

Isolation of mononuclear cells from peripheral blood was achieved by density centrifugation on a Ficoll gradient (specific density 1.077 g/ml) (Biochrom Seromed, Berlin, Germany) with consecutive washing with phosphate-buffered saline supplemented with 2% bovine serum albumin (Sigma, St. Louis, MO). After controlled freezing of the cells (10 million cells/ml in culture medium; RPMI 1640, 10% fetal calf serum, 4 mmol glutamine, 50 µg/ml streptomycin, 50 U/ml penicillin; all

reagents from Biochrom Seromed) supplemented with 7.5% dimethylsulfoxide (Sigma), specimens were stored in liquid nitrogen. After thawing and washing of the cells, proliferation assays were performed with 750,000 cells/ml in culture medium in microtiter plates. Proliferation was induced by phytohemagglutinin (PHA) (2.4 mg/ml) and pokeweed mitogen (PWM) 0.8 mg/ml), both from Biochrom Seromed, or tetanus toxoid (TET) (40 IU/ml) from Behring. To assess lymphocyte proliferation, a commercially available ELISA assay (Cell Proliferation ELISA [colorimetric], Boehringer Mannheim, Mannheim, Germany) was used to detect incorporated bromodeoxyuridine.

Additionally, proliferation studies were performed in media containing increasing concentrations of diammonium chromate (from 7 to 700 ppb) to assess that concentration of chromate that reduced the proliferation capacity of the lymphocytes to half its maximum ($Cr_{[p1/2]}$). This yielded a cumulative index of the exposure of the individual to the sum of immunotoxic substances.

A tetanus vaccination was offered to those persons who had not been inoculated within the last 10 years. Finally, the vaccination response *in vivo* was determined in a subsample of 53 persons by measuring tetanus antibody concentration in the blood before and 3 weeks after vaccination using an ELISA test kit (Immunozym Tetanus, Immuno, Heidelberg, Germany).

PCDD/PCDF Levels

In correlating the immune parameters, PCDD/PCDF determinations were used that had been obtained closest to the date of clinical and laboratory investigation of each person. If no PCDD/PCDF level was available for the date of clinical investigation but was determined earlier or later, a PCDD/PCDF level was extrapolated to the date of clinical investigation using a linear first order kinetic and the results of the half-life evaluation of Flesch-Janys et al. (*29*). It was possible to obtain PCDD/PCDF concentrations for 187 persons. Serum specimens of 135 persons taken at the time of investigation were evaluated for PCDD/PCDF contents at the Centers for Disease Control and Prevention, Atlanta, Georgia. The remaining 52 persons had PCDD/PCDF levels evaluated in whole blood (done at ERGO Forschungsgesellschaft mbH laboratories, Hamburg, Germany) or in fatty tissue (*30*) during the period 1986 to 1994. By analysis of repeated determinations done in the different laboratories a good correlation [r^2 (TEQ) = 0.8244 (O. Päpke, personal communication)] between the different methods could be achieved. TCDD TEQs were calculated according to the North Atlantic Treaty Organization Committee on the Challenges of Modern Society (*31*).

Statistics

Standard descriptive statistics were calculated to describe the study population and correlate the laboratory findings with the PCDD/PCDF levels (Pearson's and Spearman's correlation coefficient, scatterplots, two-way tables). Logistic regression was applied to analyze the immunologic parameters' dependencies on PCDD/PCDF levels in the presence of confounders (e.g., age, smoking). Significance of influence of TCDD or TEQ levels was assessed using the Wald test applied to the respective regression coefficients in the logistic regression. PCDD/PCDF concentrations were subdivided into four categories (background, moderate, high, extremely high). Each category was analyzed as a categorical independent variable and its association with a dichotomized or categorical outcome variable assessed by the χ^2 test for contingency tables. Dichotomization used standard normal ranges of these outcome variables as given by the respective laboratories. Statistical analysis was performed with the SAS computer software package 6.03 (SAS Institute, Cary, NC). The Mann–Whitney U test was used to compare the 29 highly exposed workers with their controls.

Results

Infectious Diseases

No statistically significant correlation ($p < 0.05$) was observed in this analysis for PCDD/PCDF exposure and the frequency of infectious diseases (Table 2) when using logistic regression. Only infections of the lung and fungal infections of the skin showed some borderline correlation ($0.05 < p < 0.10$).

Basic Laboratory Parameters

Of the variables of the full blood count, ESR, serum electrophoresis, IgA, IgG, and IgM, none were significantly correlated with exposure to PCDD/PCDF using the χ^2 test.

Tetanus Vaccination

The absolute and the relative rise of the concentration of tetanus antibodies 3 weeks after vaccination was calculated. No correlation between TCDD or TEQ levels and the rise of tetanus antibodies was detected (Figure 1).

Table 2. Incidence of infectious diseases during and after occupational contact with PCDD/PCDF correlated with TCDD/TEQ concentrations and analyzed by logistic regression.

Organ infected or type of infection[a]	*p*, Logistic regression
Ear	0.77
Paranasal sinus	0.35
Bronchus	0.24
Lung (pneumonia)[b]	0.06*
Influenza	0.63
All airways	0.59
Eye	0.85
Skin	0.21
Skin (fungal)	0.09
Kidney	0.46
Tuberculosis	0.27
Zoster	0.86
Worms	0.83
After an operation	0.86

[a]Occurrence of infection denoted by ($y = 1$). The model $P(y=1) = (1 + \exp(\alpha \times \text{TCDD} + \beta \times \text{confounder})^{-1}$ is analyzed statistically where α denotes the regression coefficient of the influence of TCDD/TEQ and p (logistic regression) describes its significance of influence. [b]Occurrence of pneumonia was not dependent on smoking status. *$p = 0.83$.

Autoantibodies

No correlations with TCDD or TEQ levels were apparent for the several single specific autoantibodies or for the sum of scores. There was no correlation of natural autoantibodies to PCDD/PCDF exposure.

Monoclonal Antibodies and L-Chains in Urine

L-Chains were found in the urine of two persons and monoclonal antibodies in serum were found in 11 persons. TCDD concentrations of the two persons with L-chains in urine were 16.7 and 3.8 pg/g fat, and the respective TEQ values were 49.7 and 30.9 pg/g fat. There was no correlation between the frequency of monoclonal antibodies and the PCDD/PCDF lipid concentration. The occurrence of monoclonal antibodies did not depend on age.

Lymphocyte Subgroups

A statistically significant correlation was observed between $CD3^+/CD8^+$ (T suppressor or cytotoxic T cells) and logTEQ ($p < 0.025$), respectively, a trend in $CD3^+/CD25^+$ (activated T cells) and logTCDD ($p < 0.07$) and $CD3^+/MHC$ class II^+ (activated T cells) and logTEQ ($p < 0.10$). All the regression coefficients in the logistic model were negative, indicating a decrease in cell count with increasing TCDD or TEQ levels. All other subgroups,

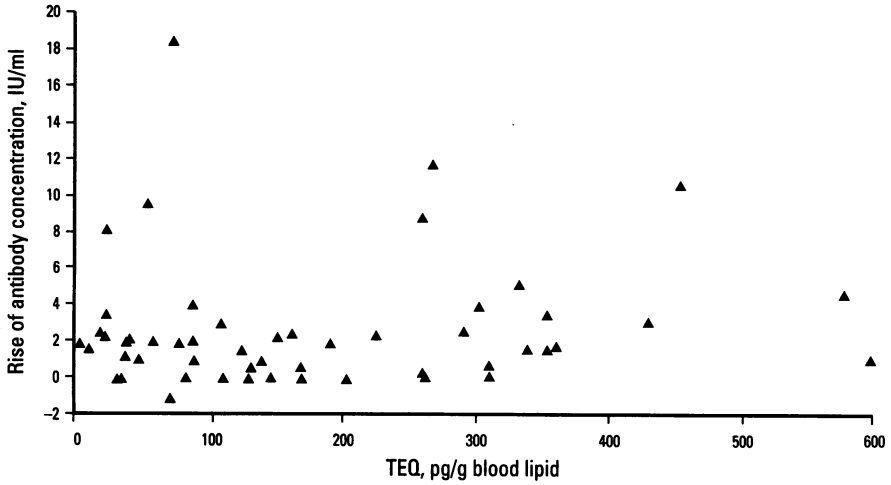

Figure 1. Rise of the concentration of tetanus antibodies three weeks after vaccination dependent on the concentration of PCDD/PCDF (expressed as TEQ) in blood lipids.

namely the $CD19^+$ (B cells), $CD19^+/CD23^+$ (activated B cells), $CD3^+/CD4^+$ (T-helper cells), $CD3^+/CD16(56)^+$ ($CD3^+$-killer cells), and $CD3^-/CD16(56)^+$ (natural killer cells) did not correlate with the actual TCDD or TEQ level.

Proliferation Studies

There was no correlation between the proliferation of lymphocytes under the influence of PHA, PWM, or TET and PCDD/PCDF concentrations. Also, there was no difference between the S_H and S_C groups under PHA and PWM stimulation. Proliferation under TET stimulation was slightly higher ($p < 0.1$) in S_H than in S_C. This difference was significant, however, only in the subgroup of persons who had undergone tetanus vaccination within the last 10 years (Figure 2A).

Chromate Resistance

No dependency of $Cr_{(p1/2)}$ in PHA-, PWM-, or TET-stimulated lymphocytes on PCDD/PCDF levels in blood was observed in the entire group of 192 persons.

PHA-stimulated lymphocytes of exposed persons tolerated significantly less chromate than those of the controls. Tolerance of chromate was higher in S_H than in S_C persons when stimulated with TET. Again this difference was significant because of the persons that had undergone tetanus vaccinations within the last 10 years (Figure 2B).

Discussion

Study Group

To our knowledge our study is the largest study to date on the impact of PCDD/PCDF on the immune system in occupationally exposed persons in which the amount of exposure is known from biomonitoring individual blood levels. Because of the complexity of the immune system, we used a broad panel of immune parameters to obtain a valid assessment of possible toxic effects on the immune system. Therefore, *in vitro, ex vivo, in vivo,* and clinical parameters were used.

A shortcoming of the study is the observational nature of the investigated group. The investigation was part of a social program offered to former plant workers and was focused primarily on the health status of individuals and their support rather than that of the entire group. Therefore, self selection cannot be excluded but appears improbable. Selection occurred because many highly exposed persons were no longer available for medical examination, as the high exposure occurred in the 1950s and some persons had died or were not able to participate in the investigation. Nevertheless, PCDD/PCDF blood levels available for most of the investigated persons provided a powerful tool to gain insight into alterations of the human immune system brought about by exposure to PCDD/PCDF.

Coexposure

Often in occupational and environmental medicine there are many different toxins that may influence the immune system at the same time. Therefore, determining only one exposure parameter might not be sufficient for analyzing a cause–effect relationship if the parameter investigated is not of outstanding influence. Even in the Boehringer cohort, where it is obvious that PCDD/PCDFs are or were present in toxic concentrations, other toxins such as β-hexachlorocyclohexane and benzene might have had an important influence on the immune system. These toxins are less well documented than PCDD/PCDF and therefore their effects can only be roughly estimated, based mostly on the predominant workplace of the persons under investigation. This possible confounding must be accepted in this occupational study as in many other studies in which only limited means are present to characterize exposure completely.

Diseases and Basic Laboratory Parameters

No relationship between PCDD/PCDF exposure and infectious diseases, except for a more frequent occurrence of acute appendicitis, was found in a morbidity study by Zober et al. (*15*). Data on the persons under investigation were gained from health insurance forms. This method of data collection excluded the influence of memory and focused on diseases that led to absences from work and that were well defined, such as appendicitis. The data were derived from a questionnaire and an interview. Especially for common infectious diseases, anamnesis depends largely on memory and on the importance the person attaches to the disease. Therefore, the borderline correlation of PCDD/PCDF concentration in blood lipids and reported infections of the lung and skin should be interpreted cautiously.

Basic laboratory parameters did not reveal any influence of the PCDD/PCDF body load. This is in agreement with previous findings that showed diverging results in the blood count and in the concentrations of immunoglobulins in serum (*16–19,21–23,26*).

Autoantibodies

Only scattered information exists about a causal relationship between PCDD/PCDF and the development of autoantibodies. Two cross-sectional studies (*19,23*) detected increased concentrations of immune complexes and ANA in exposed workers and in inhabitants of Vietnam. These findings were not confirmed in larger controlled studies. In our study no significant correlation of any single antibody or the sum of autoantibodies and PCDD/PCDF concentrations in blood lipids was found.

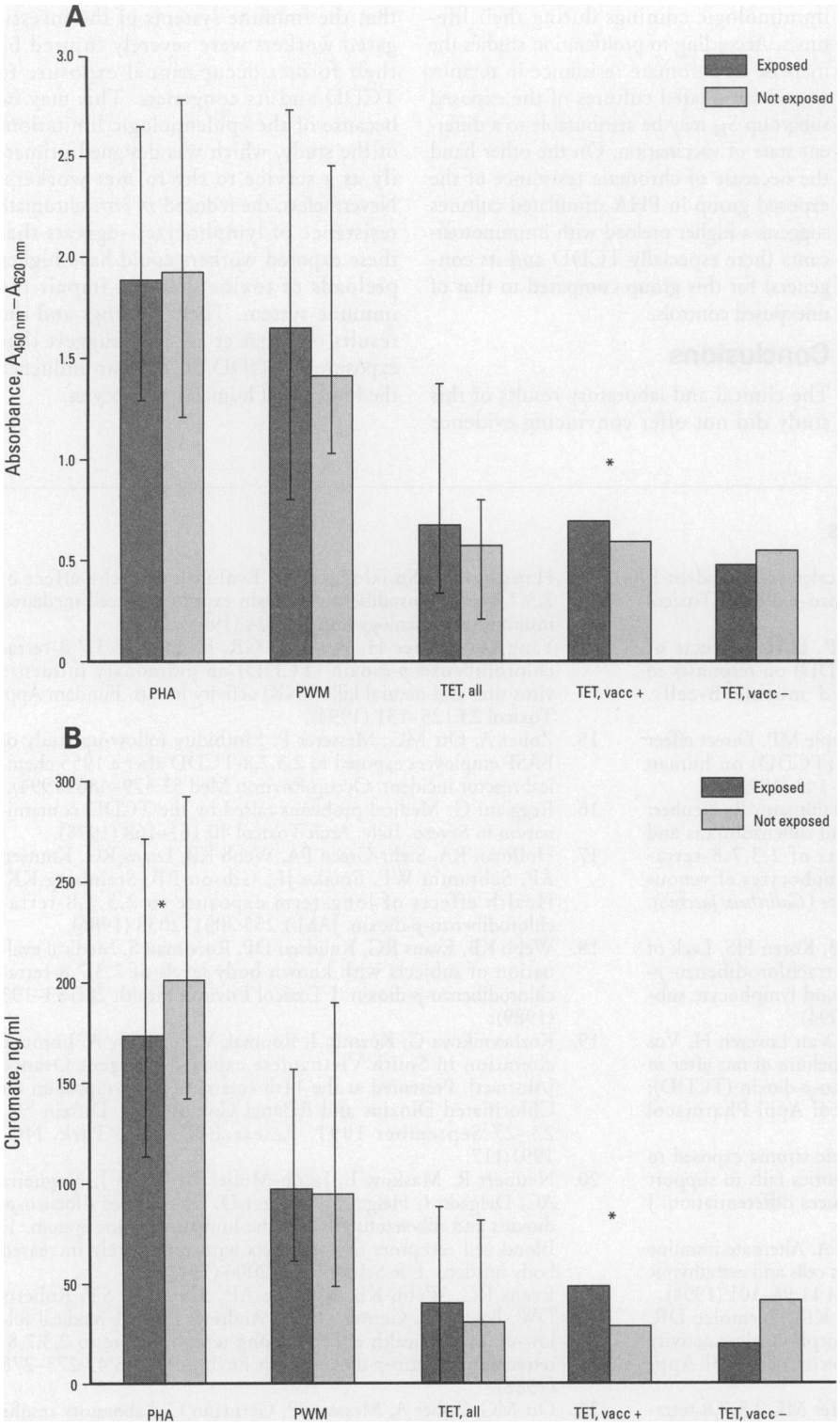

Figure 2. (*A*) Lymphocyte proliferation rate (measured as the uptake of bromodeoxyuridine, expressed as optical density) under the influence of different stimulants i.e., PHA, PWM, TET. (*B*) *In vitro* chromate resistance of lymphocytes under the influence of different stimulants. The concentration of ammonium dichromate in culture medium that reduced the lymphocyte proliferation rate to half of its maximum ($Cr_{[p1/2]}$) was determined. Medians and 5 to 95% confidence intervals are given for *A* and *B*. *$p<0.05$. Differences are given for the group of persons exposed to PCDD/PCDFs ($n=29$) and a control group of persons not exposed to PCDD/PCDFs ($n=28$). Results of the proliferation study stimulated with TET are further subdivided in those persons who were vaccinated within the last 10 years (vacc +) and those who were not (vacc –). For statistical analysis the Mann–Whitney U test was used.

Tetanus Vaccination

No effect was observed in this study of PCDD/PCDF on the rise of tetanus antibodies after vaccination. This finding fits well with the results of a study on marmosets (*32*), where no impact of TCDD on the booster effect of tetanus vaccination on tetanus toxoid stimulated lymphocyte proliferation was demonstrated. Further analyses regarding the vaccination status and other influential factors are to be carried out.

Surface Markers

Our data showed a significant negative correlation of $CD3^+/CD8^+$ cells and logTEQ and borderline significant negative correlations of $CD3^+/CD25^+$ cells and logTCDD and $CD3^+$/MHC class II^+ cells and logTEQ in the group of 192 workers. A study by Ernst et al. (*33*) showed that in a subgroup of highly exposed workers there is a positive correlation of $CD3^+/CD8^+$ cells as well with TCDD and TEQ levels that are backcalculated to the end of external exposure and exhibit a positive correlation with actual levels of exposure. Reasons for this apparent contradiction between the two studies may be statistical in origin because in our study there are few highly influential points that determine the direction of the regression slope. A second explanation is the difference in coexposures; i.e., the subgroup investigated in Ernst et al. (*33*) mainly worked in the trichlorophenol and 2,4,5-T departments, where TCDD was almost the only toxin to which they were exposed, whereas in the entire group of 192 persons many were exposed to high amounts of other toxins such as benzene and α-, β- and γ-hexachlorocyclohexane.

Proliferation Studies

In the Ernst et al. (*33*) study it is shown that there is an important difference in the activation response of lymphocytes (measured by the release of interferon-γ [IFN-γ]) on TET of whole blood cultures and those of isolated mononuclear cells. Lymphocytes of exposed persons in a culture of isolated mononuclear cells produced more IFN-γ than those of control persons; in whole blood cultures, however, the lymphocytes of the exposed persons produce significantly less. Ernst et al. (*33*) suggest a strong impeding influence of serum on the interaction of T lymphocytes and the antigen-presenting cells. As in the Ernst et al. (*33*) study, in our cultures of isolated mononuclear cells of the same

subgroups proliferation response on tetanus toxoid is higher in the exposed group than in the nonexposed group; this is probably because of the groups' different vaccination status.

Chromate Resistance

The chromate resistance test is designed to yield a cumulative factor of the immunotoxic preload of lymphocytes. The deficiency of chromate tolerance of lymphocytes *in vitro* should correlate with the donor's *in vivo* burden of immunotoxic substances. Additional influences are genetic preconditions, as different persons may have different capacities for immunotoxins, and immunologic coinings during their lifetimes. According to proliferation studies the increase of chromate resistance in tetanus toxoid-stimulated cultures of the exposed subgroup S_H may be attributable to a different state of vaccination. On the other hand the decrease of chromate resistance of the exposed group in PHA-stimulated cultures suggests a higher preload with immunotoxicants (here especially TCDD and its congeners) for this group compared to that of unexposed controls.

Conclusions

The clinical and laboratory results of this study did not offer convincing evidence that the immune systems of the investigated workers were severely injured by their former occupational exposure to TCDD and its congeners. This may be because of the epidemiologic limitations of the study, which was designed primarily as a service to the former workers. Nevertheless, the reduced *in vitro* chromate resistance of lymphocytes suggests that these exposed workers could have higher preloads of toxins that can impair the immune system. These findings and the results of Ernst et al. (*33*) suggest that exposure to PCDD/PCDFs can influence the function of human lymphocytes.

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