Persistence of Decreased T-Helper Cell Function in Industrial Workers 20 Years after Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

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In experimentally exposed animals, 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD) causes severe immunosuppression. However, the overall susceptibility of humans for the different pathological effects of TCDD has remained unclear. We examined the long-term effects of TCDD in 11 industrial workers who were exposed to high doses of TCDD for several years 20 years ago. Current TCDD body burdens were still at least 10 times higher (between 43 and 874 pg/g blood fat) in these exposed persons than in the average German population. To evaluate possible TCDD-induced changes in the percentage of different lymphocyte subsets, we determined a large panel of lymphocyte subsets in the blood by flow cytometric analysis. Immunocompetence of T- and B-lymphocytes was tested by mitogen (phytohemagglutinin, pokeweed mitogen)-induced lymphoproliferation assays and by assays using sensitive mixed-lymphocyte cultures. No significant differences could be detected between the individuals tested and controls for surface marker distribution or mitogen-induced lymphoproliferation. TCDD-exposed subjects showed a reduced response to human lymphocyte antigen-allogeneic lymphocytes and interleukin-2-boosted proliferation. Responder cells of the dioxin-exposed persons proliferated less in response to irradiated stimulator cells ($p \leq 0.05$), and the "third-party" mixed lymphocyte reaction against unirradiated stimulator cells revealed suppressive activity in the responder cell fraction compared to the controls ($p \leq 0.01$). Furthermore, the capacity of a pool of T-cells isolated from TCDD-exposed subjects to proliferate upon interleukin-2 stimulation was significantly diminished ($p \leq 0.05$). TCDD has a long-term immunosuppressive effect on T-helper cell function, which is mediated more likely by a reduced functionality of individual cells rather than by a reduction in absolute cell numbers in the peripheral blood. *Key words*: allogeneic response, immunosuppression, lymphocyte subsets, mitogen stimulation, 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin. *Environ Health Perspect* 104:422–426 (1996)

Halogenated aromatic hydrocarbons (HAHs) are ubiquituous in the environment. The most toxic and best-studied of these compounds is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Numerous studies on the toxic effects of TCDD have been done, most of them on rodents. TCDD is tumor promoting, teratogenic, and embryotoxic (*1*). One of the earliest and most sensitive markers of TCDD toxicity in experimental animals is impairment of the immune system, which is evident at doses that do not lead to overt signs of general toxicity. TCDD leads to atrophy of lymphoid organs, such as the thymus, spleen, and lymph nodes (*2*). Moreover, TCDD was shown to suppress cellular and humoral immune functions in experimental animals (*2*). Using a variety of *in vivo* exposure schemes or *in vitro* assays, TCDD was found to impair cytotoxic T-lymphocytes and natural killer cell functions, or inhibit antibody production by B-cells, for example (*2*). The capacity to respond to mitogenic stimuli, such as phytohemagglutinin (PHA) and lipopolysaccharide (LPS), is also affected by TCDD (*3,4*).

Susceptibility to the toxicity of TCDD is genetically determined by the aryl hydrocarbon receptor (*AhR*) locus. This gene codes for a cytosolic, TCDD-binding protein that is activated to a DNA-binding state upon ligand engagement (*5*) and induces the expression of a gamut of genes, including *CYP1A1*, genes of fatty acid metabolism, cytokines, and/or growth and differentiation factors (*6*). The binding affinity of AhR to TCDD and thus susceptibility varies between different animal species and also interindividually in outbred populations. Neither the overall toxicity of TCDD to humans nor TCDD-induced effects on the human immune system is known.

We examined 11 workers with defined TCDD body burdens who had been inadvertently exposed to TCDD for several years. We evaluated possible deviations in peripheral blood lymphocyte subsets using multi-parameter immunofluorescence of relevant markers (*7*) and examined *in vitro* effects of TCDD on immunocompetence of T- and B-cells.

Materials and Methods

Eleven workers, 45–63 years of age, participated in this study. They had been exposed to high doses of TCDD and other polychlorinated dibenzo-*p*-dioxins between 1966 and 1976 during production and maintenance operations at a chemical factory producing 2,4,5-trichlorophenol. Six other exposed workers declined to participate. The level of TCDD in blood lipids was determined in 1989 and 1992 by the ERGO-Forschungsgesellschaft, Hamburg, Germany, or Bioscientia, Moers, Germany, respectively, according to standard mass spectrometry (*8*). Ten age-matched, healthy, males with no known TCDD exposure history, working in the same company in office jobs, volunteered as controls. Informed consent was obtained from all subjects.

Peripheral blood (50 ml) was drawn by venipuncture into a sterile tube, containing 100 U of heparin/ml blood. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density centrifugation.

For single or dual fluorescence analysis, aliquots of 1 x 10^6 peripheral blood lymphocytes were spun down and incubated for 10 min at 6–8°C with fluoresceinated antibodies as recommended by the manufacturer. Cells were washed two times after staining, and data from 10,000 cells were collected immediately in list-mode on a FACScan flow cytometer, using appropriate compensation settings (Becton-Dickinson, Mountain View, California). Forward and rectangular light scatter gates were used.

The following fluorochromed antibodies were used: mouse anti-$CD3^{FITC}$ (clone SK7), mouse anti-$CD4^{FITC}$ (clone SK3), mouse anti-$CD8^{PE}$/$CD8^{FITC}$ (clone SK1), mouse anti-$CD19^{FITC}$ (clone 4G7), all from Becton-Dickinson; mouse anti-$CD45RO^{PE}$ (clone UCH-L1) and anti-$CD45RA^{PE}$ (clone F8-11-3), both from Serotec (Oxford,

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We appreciate the cooperation of the participants in this study. We thank Zhi-Wei Lai for critically reading the manuscript, Swantje Steinwachs and Dörte Post for technical help, and Britt Harms for help with the statistics. This study was supported in part by grant 01 KD 89030 from the Bundesministerium für Forschung und Technologie, Germany. The work of C.E. and E.G. is supported through SFB 503, "Molecular and cellular mediators of exogenous noxes" at the Heinrich-Heine-University of Düsseldorf.

Received 2 October 1995; accepted 2 January 1996.

UK); mouse anti-CD56^{PE} (clone B 159.5; Pharmingen, San Diego, California); and anti-CD57 (Leu7; Becton-Dickinson).

We cultured 5×10^4 cells/well in 96-well round-bottom plates containing 150 µl RPMI 1640 culture medium, supplemented with 5% heat-inactivated pooled human AB0 serum, L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential amino acids, 50 U/ml penicillin, and 50 U/ml streptomycin.

Cells were cultured in triplicate either with medium alone or in the presence of 9 µg/ml PHA (Sigma, St. Louis, Missouri) or 6 µg/ml pokeweed mitogen (PWM; Sigma) for 3 days at 37°C in a water-saturated 5% CO_2 atmosphere. On day 4 of culture, cells were pulsed with 1 µCi of ^{3}H-thymidine and then harvested 20 hr later to determine incorporated radioactivity (ß counter LS 6000 IC, Beckman Instruments, München, Germany).

The human lymphocyte antigen (HLA) phenotype of peripheral blood mononuclear cells was determined by standard microlymphotoxicity assays using A,B,C plates (One-lambda, Canoga Park, California) and HLA-class II plates (Biotest, Dreieich, Germany). For mixed lymphocyte cultures, washed cells were resuspended in serum-free CG-medium (Vitronex, Vilshofen, Switzerland), supplemented with 60 µg/ml gentamcycin. We seeded 5×10^4 responder cells in triplicate into 96-well round-bottom microtiter plates. For "one-way" mixed lymphocyte cultures, the same number of irradiated (30 Gy) stimulator cells were added. Stimulator cells were from a pool of cells from 20 allogeneic, HLA-unrelated individuals. For "third-party" suppressor assays, the lymphocytes from dioxin-exposed or control persons were irradiated (30 Gy) and added to the pooled allogeneic cells (in this case unirradiated).

Interleukin-2 (IL-2)-inducible proliferation of T-cells was measured after adding 30 U/ml recombinant IL-2 per 5×10^4 cells. Recombinant IL-2 was a gift of P. Loeliger (Sandoz, Switzerland).

Results

The concentration of TCDD in the blood of 11 workers exposed between 2 and 11 years before 1976 (with one exception, see Table 1) was determined in 1989 or 1992, i.e., 13–15 years after the last exposure. Table 1 summarizes the exposure parameters and the health status of the TCDD-exposed workers determined at a thorough general medical examination in 1992. The TCDD values in blood fat differed up to 20-fold between individuals, yet even the lowest burdens were well above the average level of the German population, which is about 4 pg/g blood fat (*9*). At the time of the study, five persons still suffered from chloracne, of which one had chronic gastritis and one hyperthyreosis. Two subjects displayed a disturbance of fatty acid metabolism. The others appeared healthy.

The frequencies of various lymphocyte subsets were determined by flow cytometry. The percentages of B-cells (CD19), T-cells (CD3), and subsets thereof were determined, e.g., T-helper cells (CD4), cytotoxic T-cells (CD8), virgin helper/inducer T-cells (CD4CD45RA) as well as primed helper/inducer (CD4CD45RO). Moreover, natural killer cells (CD56 and CD57) and HLA-DR expression were measured. Some of the subset markers were previously shown to be sensitive parameters of TCDD-exposure in marmosets, a new world primate species (*7*). Table 2 shows the result of the analysis for each of the 11 TCDD-exposed individuals and for the controls. No difference between controls and the mean values of the TCDD-exposed group was evident for any of the lymphocytes subsets analyzed. Note that the per-

Table 1. TCDD exposure length, severity, and health status[a]

Subject no.	Smoker	Years of exposure	TCDD in blood fat (pg/g)	Year of exam	Chloracne	Clinical manifestations
1	Yes	1966–76	874	1989	Yes	Chronic gastritis
2	No	1966–76	274	1992	Yes[b]	Hyperthyreosis
3	No	1973–76	264	1989	No	None
4	Yes	1971–74	190	1989	No	Disturbance in fatty acid metabolism
5	Yes	1974–76	54	1992	No	Disturbance in fatty acid metabolism
6	No	1981–82	90	1989	No	None
7	No	1971–76	43	1992	No	High incidence of colds
8	Yes/No[c]	1963–76	720	1992	Yes	None
9	No	1972–76	95	1989	No	None
10	No	1967–74	287	1989	Yes[d]	None
11	No	1973–75	734	1992	Yes	None

[a]The concentration of 2,3,7,8-TCDD in the blood fat of 11 workers who had been exposed for different intervals between 1966 and 1981 to 2,3,7,8-TCDD and related congeners was determined, and a general medical examination was done in 1989 or 1992, at the same time.
[b]Chloracne from 1974 to 1981.
[c]Past smoker.
[d]Chloracne for a short period in 1974.

Table 2. Lymphocyte subsets in peripheral blood mononuclear cells of 11 industrial workers and controls

Parameter	Subject no. 1	2	3	4	5	6	7	8	9	10	11	Mean ± SD	Controls (n=10) Mean ± SD
Age (years)[a]	58	63	57	58	46	45	56	61	46	49	50	53.5	54.4
2,3,7,8-TCDD level (pg/g blood fat)	874	274	264	190	54	90	43	720	95	287	734	329.5	ND
Lymphocyte subsets[b]													
CD3	76.9	68.8	76.1	71.7	66.7	55	76.7	63.8	64.5	81.0	74.4	70.5 ± 7.6	70.9 ± 10.5
CD4	50	51	61	47	47	41	43	49	44	52	38	47.6 ± 8.1	48.5 ± 10.6
CD8	25	17	21	27	24	20	30	15	21	27	34	23.7 ± 5.6	22.1 ± 3.9
CD19	10.3	5.9	5.7	6.2	5.2	12.2	8.6	13.1	7.2	6.1	2.0	7.3 ± 3.2	6.8 ± 3.4
CD56	3	5	5	5	6	9	5	4	9	4	5	5.4 ± 1.9	5.5 ± 1.6
CD57	12	15	19	20	11	15	30	10	21	17	35	19.4 ± 8.3	20.0 ± 7.5
CD4 CD45R0	12	18	12	7	11	10	15	22	14	5	11	12.4 ± 4.8	11.0 ± 4.5
CD4 CD45RA	18	13	18	16	20	14	13	6	ND	15.7	7	16.2 ± 5.5	12.0 ± 6.0
CD3 HLA-DR	3	0	2	5	4	4	4	3	5	7	19	5.1 ± 4.9	4.5 ± 2.5

ND, not determined.
[a]Lymphocyte subsets were determined in cells of TCDD-exposed persons versus unexposed, age-matched controls. The age given refers to the age at the time of TCDD determination (see Table 1).
[b]Values are percentage of total, ficollated peripheral blood mononuclear cells.

centage of CD56-expressing cells (i.e., natural killer cells) is comparatively lower than reported in the literature for younger individuals, possibly a phenomenon of age (*27*).

Peripheral blood lymphocytes were tested for their capacity to respond to mitogens PHA and PWM. As shown in Figure 1, lymphocytes from TCDD-exposed and control persons responded equally well to mitogen stimulation, with some individual variation. There was no statistically significant difference between the response of the two groups (p = 0.5641). Moreover, when we corrected the data for the interindividual differences in T-cell frequencies in the blood (see Table 2), it becomes even more clear that TCDD exposure does not affect the mitogen-induced proliferative capacity (p = 0.7823; data for PHA stimulation not shown). No correlation existed between individual TCDD levels in the blood nor the age of the persons and the respective proliferative capacity of their lymphocytes (not shown).

We tested the capacity of T-cells ("responder cells") from dioxin-exposed persons to specifically react against irradiated, HLA-different, allogeneic lymphocytes ("stimulator cells") in a mixed-lymphocyte culture. In another experiment, we added irradiated responder cells as a third party to a pool of unirradiated peripheral blood mononuclear cells from 20 different donors. Whereas the former assay measures the response to allo-major histocompatibility complex, the latter is used to detect suppressive factors/cells in the responder cell population, which would inhibit the respective response of the unirradiated cells (*10*).

As shown in Figure 2, the responder cells of the TCDD-exposed persons proliferated less in response to irradiated stimulator cells (p<0.05 by Student's t-test). Moreover, the third-party mixed lymphocyte reaction against unirradiated stimulator cells revealed a small amount of suppressive activity in the responder cells of dioxin-exposed individuals, resulting in a decreased overall proliferation of T-cells (Fig. 2,). This significant suppression (p <0.01 by Student's t-test) is indicative of a reduced T-helper cell response (*11*). However, the actual number of T-helper cells was unaffected by TCDD (see Table 2).

The capacity of a pool of T-cells to proliferate upon IL-2 stimulation is a parameter of normal T-cell function and correlates with the presence of preactivated T-cells in the pool, which would result in a higher overall proliferative response. Peripheral blood mononuclear cells of TCDD-exposed individuals and control persons were co-cultured with a low dose of IL-2 for 4 days. The cells of TCDD-exposed persons revealed a reduced capacity to proliferate with IL-2 (Fig. 2; p<0.05). The values for one exposed individual were not included in the data due to an excessively high proliferation rate. Those particular values reflected an extreme preactivation of the T-cells, which is typical for the beginning of an acute, but undetected, infection. No atopic status is known for that person. However, it must be noted that the statistically significant differences between the TCDD-exposed and control persons disappear if this one value is included.

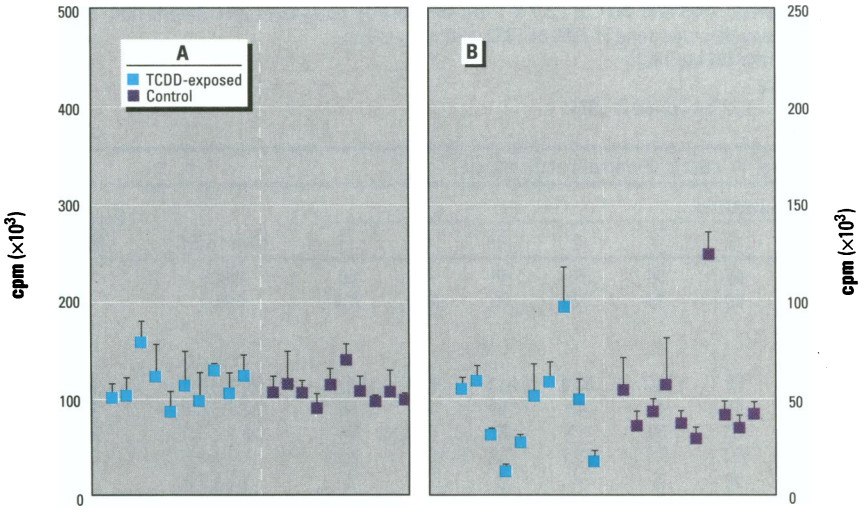

Figure 1. Proliferation capacity of (A) peripheral blood T-cells in the presence of phytohemagglutinin and (B) T-cells and B-cells in the presence of pokeweed mitogen in individuals exposed to dioxins about 20 years ago. We cultured 1×10^5 Ficoll-isolated peripheral blood mononuclear cells in the presence of mitogen for 4 days, and pulsed with ^{3}H-thymidine 20 hr before harvesting. Shown are the means and SDs (bars) of triplicate cultures (TCDD exposed and controls). The order of symbols for dioxin-exposed persons are arranged from 1 to 7 and 9 to 11 as they appear in Tables 1 and 2; the value for person 8 was not determined.

Discussion

Ever since the major accident at Seveso, Italy, the danger of dioxin has been recognized. The acute toxicity dose of TCDD is among the lowest for any known chemical substance. Fortunately, no people died at Seveso, and most scientists believe humans to be at the lower end of the susceptibility scale. However, TCDD continues to be inadvertently released into the environment, and little is known on the long-term effects of low doses. In Germany, the body burden of the general population is about 4 ppt TCDD in blood fat, with an estimated daily uptake, mostly by food, of 26 pg (*9*).

The immune system seems to be the most sensitive target of TCDD action in experimental animals. Humoral as well as cellular components of immune responses are suppressed by TCDD (*2,12,13*). The interaction of the human immune system with TCDD has remained controversial. Studies with an evolutionarily closely related species, the primate *Callithrix jacchus* (marmosets), revealed sensitive parameters for TCDD-induced alterations in peripheral blood lymphocyte subsets. Using combina-

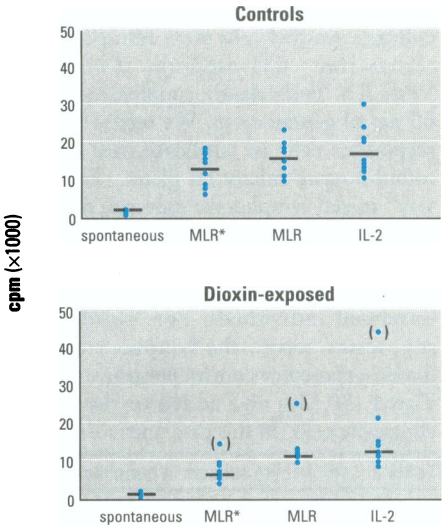

Figure 2. Proliferation of peripheral blood mononuclear cells (PBMCs) of dioxin-exposed persons and controls. Tested were 1) the spontaneous response, 2) the response toward irradiated stimulator PBMCs of unrelated HLA-haplotypes (MLR), 3) the third-party response of irradiated PBMCs of TCDD-exposed persons toward the mixed-lymphocyte reaction of the pool of unirradiated stimulator cells (MLR*), and 4) the proliferative capacity in response to interleukin-2 stimulation (IL-2). Cultures were done in triplicate. Each dot represents the mean of the count per minute values of the PBMCs of one person. For p-values from Student's t-test, see text. One dioxin-exposed person (value in parentheses) displayed a very high response in all assays, probably indicating preactivated T-cells (e.g., due to an undetected, ongoing infection). This value was disregarded for statistical calculations (see text).

tions of surface markers, changes in the frequency of memory ($CD4^+$, $CDw29^+$), suppressor-inducer ($CD4^+$, $CD45RA^+$) T-cells and B-cells ($CD20^+$) were found (*7*). *In vitro* studies on human blood leukocytes after accidental or occupational exposure to TCDD were ambiguous with respect to the immunological alterations induced (*14*). Some authors reported suppressed responses (*15*), whereas others reported enhanced mitogen-induced lymphoproliferative responses (*16*) and increased percentages in suppressor/cytotoxic T-cells and the absolute number of natural killer cells in the peripheral blood (*17,18*). Others found no effect on mitogen stimulation (*19*) and no deviations in peripheral blood subsets (*20,21*). With the exception of one of these studies (*21*), the actual body burden had not been determined. It is interesting to note that also in experimental animals the immunosuppressive effects of TCDD are commonly detected by functional tests (i.e., following antigen-specific stimulation), rather than unspecifically, by mitogen stimulation, for example. Thus, it was important to choose an appropriately sensitive test system.

We tested possible immunosuppression in TCDD-exposed workers using three parameters of lymphocyte competence. We found no difference compared to the age-matched control persons for the distribution of lymphocyte subsets in the blood. This result is in accordance with the more elaborate study of Neubert and co-workers (*7*), in which they applied the experience gained in *Callithrix jacchus* on TCDD-sensitive lymphocyte subsets to humans, but failed to detect any decrease in leukocyte subsets associated with elevated dioxin body burdens. In the present study, we demonstrated a small but significant difference in the allo-response of T-cells and in their proliferative response to IL-2. Moreover, the lymphocytes of TCDD-exposed persons displayed a suppressive activity, which inhibited an ongoing allo-response of HLA-unrelated lymphocytes. This is probably due to an increased proliferation response of T-cells counteracting the $CD4^+$ T-helper-1 cells, representing the primary responder type in mixed lymphocyte cultures.

As reported previously (*22*), the changes in immunocompetence we observed *in vitro* did not correlate with obvious diseases related to severe immunodeficiency such as certain cancers and infections. The workers were generally healthy and, with one exception, had no history of increased susceptibility to infections. The functional reserve of the immune system is enormous, so that impaired immune responsiveness need not have pathological consequences (*23*). Indeed, the term "immunotoxic" has not been properly defined (*24*). Only a large, well-controlled epidemiological study might reveal the actual health effects of subtle changes in immunocompetence.

The reduced immunocompetence *in vitro* observed here fails to correlate with a reduced number of lymphocyte subsets. Thus, TCDD-induced immunosuppression is more likely mediated by a reduced functionality of individual cells rather than by a reduction in cell numbers circulating in the blood.

Thymus involution, a reduction of thymus weight and cellularity, is a hallmark of TCDD exposure. In mice, TCDD skews the distribution of lymphocyte subsets (*25,26*). However, although a link between thymus events and peripheral immunosuppresssion is often implicitly assumed, nothing is known about the balance between thymocyte/T-cell generation and migration to the periphery under TCDD treatment. The thymus normally begins to atrophy at about the time of sexual maturity in mice and humans (*27*). T-cell numbers and other leukocytes in the periphery remain unaffected in TCDD-exposed versus nonexposed persons (*28*), thus an additional atrophic effect on the thymus by TCDD might not be detectable in people exposed to TCDD as adults over about 30 years of age.

We found no correlation between TCDD levels in blood and performance of peripheral blood mononuclear cells in the assays. This is not surprising, since the development of chloracne, the major effect of dioxin exposure on humans, in individuals affected by the Seveso accident did not correlate to the severity of TCDD exposure. It is now generally accepted that the Ah receptor mediates dioxin toxicity, and the reason for variability in individual responses is likely due to genetic differences in Ah receptor alleles in different individuals.

We have presented here data where the actual body burden of the individuals analyzed were known. We know of only two other such studies. In accordance with our data, no phenotypic difference between lymphocyte subsets in the exposed and unexposed groups was found, with the exception of CD8 cells, which were slightly increased (*21,22*). However, the present study is the first to demonstrate reduced immunocompetence of lymphocytes from TCDD-exposed humans.

References

1. Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons; examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22:517–554 (1982).
2. Holsapple MP, Snyder NK, Wood SC, Morris DL. A review of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence:1991 update. Toxicology 69:219–255 (1991).
3. Faith RE, Luster MI. Investigations on the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on parameters of various immune functions. Ann NY Acad Sci 320:564–571 (1979).
4. Dooley RK, Holsapple MP. Elucidation of cellular targets responsible for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced suppression of antibody responses: I. The role of the B lymphocyte. Immunopharmacology 16:167–180 (1988).
5. Swanson H, Bradfield CA. The AH-receptor: genetics, structure and function. Pharmacogenetics 3:213–230 (1993).
6. Sutter TR, Greenlee WF. Classification of members of the Ah gene battery. Chemosphere 25:223–226 (1992).
7. Neubert R, Stahlmann R, Korte M, van Loveren H, Vos JG, Webb JR, Golor G, Helge H, Neubert D. Effects of small doses of dioxins on the immune system of marmosets and rats. Ann NY Acad Sci 686:662–686 (1993).
8. Patterson DG Jr, Hampton L, Lapeza CR Jr, Belser WT, Green V, Alexander L, Needham LL. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem 59:2000–2005 (1987).
9. Schrey P, Wittsiepe J, Ewers U, Exner M, Selenka F. Age-related increase of PCDD/F-levels in human blood—a study with 95 unexposed persons from Germany. Organohalogen Compounds 9:261–267 (1992).
10. Pawelec G, Wernet P, Rehbein A, Balko I, Schneider EM. Alloproliferative human T cell clones primed and cultured in vitro lose proliferative and gain suppressive activity with age. Hum Immunol 10:135–142 (1984).
11. Pawelec G, Schneider EM, Wernet P. Cloned human T lymphocytes with lymphostimulatory capacity preferentially activate suppressor cells. Eur J Immunol 14:335–340 (1984).
12. Clark D, Gauldie J, Szewczuk MR, Sweeney G. Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc Soc Exp Biol Med 168:290–299 (1981).
13. Morris DL, Snyder NK, Gokani V, Blair RE, Holsapple MP. Enhanced suppression of humoral immunity in DBA/2 mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 112: 128–132 (1992).
14. Stehr PA, Stein G, Webb K, Schramm W, Gedney WD, Donnell HD, Ayres S, Falk H. A pilot epidemiologic study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin contaminations in Missouri. Arch Environ Health 41:16–22 (1987).
15. Knutson AP. Immunologic effects of TCDD exposure in humans. Bull Environ Contam Toxicol 33:673–681 (1984).
16. Tognoni G, Bonaccorsi A. Epidemiological problems with TCDD (a critical review). Drug Metab Rev 13:447–469 (1982).
17. Kochmann S, Cazabat JBA, Lavand F, Lorton C, Rappe C. Phenotypical dissection of immunoregulatory T cell subsets in humans

after furan exposure. Chemosphere 15:9–12 (1986).
18. Jennings AM, Wild G, Ward JD, Ward AM. Immunological abnormalities 17 years after accidental exposure to 2,3,7,8-TCDD. Br Med J 45:701–704 (1988).
19. Pocchiari F, Silano V, Zampieri A. Human health effects from accidental release of tetrachlorodibenzo-p-dioxin. Ann NY Acad Sci 320:311–320 (1979).
20. Wolfe WH, Michalek JE, Miner JC. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. I. Physical health. J Am Med Assoc 264:1824–1831 (1990).
21. Zober MA, Ott MG, Päfke O, Senft K, Germann C. Morbidity study of extruder personnel with potential exposure to brominated dioxins and furans. I. Results of blood monitoring and immunological tests. Br J Ind Med 49:532–544 (1992).
22. Webb KB, Evans RG, Knutsen AP, Roodman ST, Roberts DW, Schramm WF, Andrews JS, Needham LL, Patterson DG. Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 28:183–193 (1989).
23. Neubert R, Golor G, Helge Hans, Neubert D. Risk assessment for possible effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related substances on components and functions of the immune system. Exp Clin Immunogenet 11:163–171 (1994).
24. Neubert D, Neubert R, Stahlmann R, Helge H. Immunotoxicology and -pharmacology. Braz J Med Biol Res 22:1457–1473 (1989).
25. Esser C, Welzel M. Ontogenetic development of murine fetal thymocytes is accelerated by 3,3′,4,4′-tetrachlorobiphenyl. Int J Immunopharmacol 13:841–852 (1993).
26. Lai ZW, Kremer J, Gleichmann E, Esser C. 3,3′,4,4′tetrachlorobiphenyl inhibits proliferation of immature thymocytes in fetal thymus organ culture. Scand J Immunol 39:480–488 (1994).
27. Klein J. Immunology. Oxford:Blackwell Scientific Publications, 1990
28. Neubert R, Maskow L, Webb J, Jacob-Müller U, Nogueira AC, Delgado I, Helge H, Neubert D. Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. I. Blood cell receptors in volunteers with moderately increased body burdens. Life Sci 53:2007–2018 (1993).