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Immunologic Effects of TCDD Exposure in Humans

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD),[1] a contaminant found in the manufacture of a number of useful halogenated hydrocarbons, has been shown to have marked toxicity in experimentally exposed animals (McConnell and McKinney 1978). In all animal species studied, significant thymic atrophy with cortical thymic depletion has been observed (McConnell 1980). In addition, depletion of lymphocytes in T-dependent regions of peripheral lymphoid tissue, such as the paracotical area in lymph nodes and periarterial sheath in the spleen, is uniformly observed (Vos et al. 1980; Vos and Moore 1974). These changes have been observed in both acute and chronic TCDD exposed animals and are most pronounced in neonatal animals (McConnell 1980). Subsequent studies of cellular immunity showing that T cell functions are suppressed have corroborated these histological findings. TCDD exposed mice have depressed delayed hypersensitivity skin responses, prolonged allograft rejection and increased susceptibility to certain infectious agents (Vos and Moore 1974; Vos et al. 1974). In vitro studies of isolated lymphocytes have shown depressed lymphoproliferative responses to T-dependent mitogens (Vos and Moore 1974; Vos et al. 1974; Luster et al. 1980; Faith et al. 1978) and depressed generation of cytotoxic T lymphocyte responses (Clark et al. 1981).

An intriguing observation in TCDD exposed mice has been the demonstration of different strain susceptibility to TCDD induced immunotoxicity. Poland and Glover (1980) and Vecchi et al. (1983) have shown that certain strains of mice exposed to TCDD were extremely sensitive to TCDD whereas others were less sensitive, and F_1 hybrids of the two strains had intermediate susceptibility. Further studies have demonstrated that the immunotoxicity to TCDD segregated with its ability to induce the arene hydrocarbon hydroxylase (AHH) system (Poland and Glover 1980; Vecchi et al. 1983). TCDD is first bound by a "receptor" in the cytosol of cells in many organs, including the liver and thymus, which then results in induction of AHH. Sensiti-

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vity to TCDD induced AHH activity correlated well with immunotoxic effects. In humans, Kouri et al. (1975) have shown that TCDD stimulated AHH activity in in vitro lymphocyte cultures, and others have demonstrated polymorphism of the AHH system (Kellerman et al. 1973).

B lymphocytes are also suppressed in TCDD exposed animals, though generally at higher doses than those required to suppress T cell responses (Luster et al. 1979; Vecchi et al. 1980). Lymphoproliferative responses to the B cell mitogen, lipopolysaccharide, in exposed mice are reduced in a dose dependent fashion. In vivo both primary and secondary antibody responses are decreased in TCDD exposed mice immunized to SRBC (Vecchi et al. 1980), and in vitro their plaque forming cells are also depressed (Kellerman et al. 1973). Strain susceptibility and TCDD inducibility of AHH is also seen in humoral responses similar to those seen in T cell-mediated immune responses (Poland and Glover 1980).

Vos et al. (1978) demonstrated that part of the increased mortality to infectious agents in TCDD exposed mice was related to a marked increase in sensitivity to endotoxin from gram negative bacteria. Studies to explain this phenomenon examined macrophages, which are principal cells in endotoxin detoxification. In summary, macrophage dependent functions, including phagocytosis, killing and nitroblue tetrazolium reduction of peritoneal macropahges have been found to be normal in TCDD exposed adult mice. Further studies are needed to understand this phenomenon.

Though TCDD does have toxic effects in humans (Reggiani 1978; Pazderova-Vejlupkova et al. 1981), little is known regarding effects on human immunity. Reggiani (1980) reported on the immune status of 44 children (20 of whom had chloracne) in the Seveso, Italy accident and found normal quantitative serum immunoglobulins and complement concentrations, normal percentages of T and B lymphocytes and normal lymphoproliferation to mitogens. No increased incidence of infections was reported. However, Bekesi et al. (1978) found that in Michigan dairy farm residents exposed to a related halogenated hydrocarbon, a polybrominated biphenyl (PBB), in the form of contaminated meat and dairy products there was significant suppression of cellular immunity. These subjects had decreased percentages and absolute numbers of T cells, decreased lymphocyte transformation to alloantigens and an increased percentage of lymphocytes with no detectable membrane markers. The study was repeated approximately one year later by Silva et al. (1979), who detected no abnormalities. Immune function may recover in sublethal TCDD exposed mice after several months once exposure is discontinued (Faith et al. 1978), and perhaps this explains the disparity of immune findings in the Michigan farmers.

In Feburary, 1983 the Centers for Disease Control, the Missouri Division of Health and St. Louis University Medical Center conducted a pilot medical health survey of Missouri residents exposed to TCDD

contaminated soil in the Times Beach area. Based on previous animal studies, cell mediated immunity was examined by measuring delayed hypersensitivity skin tests, T cell subsets and lymphocyte proliferative responses.

Delayed hypersensitivity skin tests (DTH) were performed by the intracutaneous application of seven recall antigens (tetanus, diphtheria, old tuberculin, proteus, streptokinase, Candida and Tricophyton) using a Multi-Test device (Merieux Institute USA, Miami, FL) (Kniker et al. 1979). Skin test sites were read at 48 hours. Both the number of positive skin tests ($\geq$ 2 mm induration) and the sum of the diameters of induration were recorded.

T lymphocyte subset analysis was performed using FITC labeled monoclonal antibodies (Ortho Pharmaceuticals, Raritan, NJ) to detect T cell surface antigens and analyzed in a Spectrum III flow cytometer (Ortho Diagnostics, Westwood, MA) (Hoffman et al. 1981; Lanier and Warner 1981). OKT3 (pan T lymphocytes), OKT4 (T inducer) and OKT8 (T suppressor cytotoxic) monoclonal antibodies were used to detect total T lymphocytes and subsets.

Lymphocyte proliferation studies were performed *in vitro* on mononuclear cells isolated from heparinized venous blood by Ficoll-Hypaque density centrifugation (Schiff et al. 1974). Mononuclear cells were cultured either with medium alone or with the mitogens phytohemagglutinin (PHA, Difco Laboratories, Detroit, MI), conconavalin A (Con A, Sigma Chemical Company, St. Louis, MO) and pokeweed mitogen (PWM, Grand Island Biologicals, Grand Island, NY) or with the antigen tetanus toxoid (Massachusetts State Health Department).

The results of the DTH skin tests are illustrated in Table 1. The patients were placed in either high or low risk TCDD exposed groups as discussed by Dr. Webb. Since earlier studies had determined less skin reactivity for adult females, we further divided the exposure groups into adults ($\geq$17 years old), male and female, and children. No statistical differences were found comparing the various high versus low exposure groups, although several trends were noted for less DTH reactivity in high TCDD exposed groups. There were fewer positive skin tests found in high risk children (2.7 vs 3.5) and in high risk adults (3.3 vs 3.9) and decreased induration in the two groups (8.7 vs 10.6 and 12.6 vs 17.1 respectively). No differences were observed in the adult female groups.

Analysis of T lymphocyte subsets revealed no significant differences of either percentages or absolute numbers of T cells (OKT3), T helper (OKT4), T suppressor cells (OKT8) or T helper/suppressor ratios (T4/%8), comparing the high versus low exposure groups (Table 2). However, two trends were noted in the high exposure group that indicated the possibility of altered T cell surface markers. There was a greater percentage of individuals with a T4/T8 ratio $\leq$ 1.0 in the high risk group compared to the low risk group (15% vs 6% respectively). In addition, using the calculation (T4 + T8)/T3 to examine the

Table 1. Delayed hypersensitivity skin test results with multi-test in subjects exposed to TCDD

	Low Risk			High Risk		
	Male	Female	Children[1]	Male	Female	Children[1]
Total Subjects	12	10	8	27	22	15
No. Anergic	0	1 (10.0%)	0	0	1 (4.6%)	0
Geom. Mean Score, mm	17.1	8.2	10.6	12.6	9.1	8.7
Mean No. Antigens Positive[2]	3.9[3] ± 2.0	2.9 ± 1.9	3.5 ± 1.8	3.3 ± 1.5	2.8 ± 1.7	2.7 ± 1.3

[1]Children less than 17 years old.

[2]Induration greater than 2 mm at 48 hours is considered a positive reaction

[3]Data expressed in the mean number of antigens positive ± 1 S.D.

Table 2. T-lymphocyte profile in subjects exposed to TCDD

	Low Risk (32)	High Risk (68)
Lymphocyte/μl	1462 ± 227	1502 ± 67
OKT3/μl	945 ± 64	1086 ± 56
percent	70.3 ± 1.3	71.6 ± 0.8
OKT4/μl	588 ± 45	652 ± 35
percent	43.5 ± 1.2	43.4 ± 1.1
OKT8/μl	338 ± 26	425 ± 30
percent	25.6 ± 1.3	27.7 ± 1.1
T4/T8 ratio	1.85 ± 0.11	1.80 ± 0.10

Data are presented as means ± S.E.M.

percentage of OKT3 cells with both T4 and T8 antigens on their surface, 7/68 (10%) of individuals in the high risk group had a ratio > 1.17 compared to none in the low risk groups, indicating the possibility of dual surface markers on OKT3+ cells. During normal maturation of thymocytes, cells express both OKT4 and OKT8 antigens, but prior to release into the peripheral blood express only T4 or T8 (Reinherz et al. 1983; Janossy et al. 1981). This unusual phenotype (OKT 3+ 4+ 8+ cells) is seen following recovery of the T lymphocyte populations in renal (Cosimi et al. 1981) or cardiac transplantation patients following cessation of anti-thymocyte antibody therapy (unpublished observation). This finding and the increased incidence of low T4/T8 ratios in highly exposed individuals suggested altered T cell surface markers. Beseki, using sheep red blood cells and complement rosetting techniques, had found an increased incidence of lymphocytes devoid of surface markers in PBB exposed Michigan residents (Beseki et al. 1978). Since TCDD is preferentially toxic to cortical thymocytes, it is feasible that altered T cell surface markers may result from release of immature T cells into the peripheral blood. Future studies, which include examination of thymocyte surface markers, need to be done to determine the significance, if any, of these observations.

Lymphoproliferative responses of cultured mononuclear cells to mitogens and antigens were performed to examine T cell function. Previous studies have shown that PHA and antigens stimulate T helper cells, Con A stimulates both T helper and suppressor cells and PWM stimulates T and B cells (Reinherz and Schlossman 1980). No statistical differences were observed comparing high and low risk groups for lymphoproliferative responses (Table 3). A trend was noted that high risk children had lower tetanus toxoid stimulated blastogenesis compared to their low risk counterparts (7,256 cpm vs 42,485 cpm).

In summary, we observed no statistically significant alterations of cellular immunity in Missouri residents exposed to TCDD over a long

Table 3. Lymphocyte proliferative responses in subjects exposed to TCDD

Exposure		^{3}H-thymidine incorporation, cpm			
		PHA	Con A	PWM	Tetanus
High	Adult	63,834[1] 48.81 +.18[2] 29[3]	48,124 4.68 + .20 28	37,540 4.57 + .21 29	12,979 4.11 + .69 29
	Children	83,878 4.92 + .18 8	68,188 4.83 + .20 8	41,053 4.61 + .17 8	7,256 * 3.86 + 104 8
	Group	67,717 4.83 + .18 37	51,999 4.72 + .20 36	38,278 4.58 + .20 37	11,446 4.06 + .77 37
High	Adult	58,261 4.77 + .19 15	44,370 4.65 + .17 13	34,014 4.53 + .25 14	7,077 3.5 + .71 15
	Children	81,340 4.91 + .14 5	58,482 4.77 + .19 5	52,643 4.72 + .12 5	42,485 * 4.63 + .20 5
	Group	63,330 4.80 + .19 20	47,908 4.68 + .18 18	38,157 4.58 + .23 19	11,084 4.04 + .70 20

[1]Mean cpm of optimum response

[2]Log geometric mean cpm ± 1 S.D.

[3]Number in each group

period of time, which was comparable to the studies of Reggiani. However, several trends were noted which suggested that perhaps TCDD does affect human CMI. There were decreased DTH responses in children and adult males and decreased lymphoproliferative responses in tetanus toxoid in children. There was also evidence for altered T cell surface markers as indicated by a greater percentage of high risk subjects with low T4/T8 ratios and by an increased percentage of individuals with T3+ T4+ T8+ cells. Is TCDD responsible for these trends? Immunotoxicity in humans exposed to TCDD might be expected to occur extrapolating from the animal data and from Bekesi's study. Likewise, we might also expect varying sensitivity to TCDD immunotoxicity, which seems to be linked to TCDD inducibility of AHH in animal models. Certainly, there are also differences of inducibility of the AHH system in humans (Kellerman et al. 1973). The route of exposure might be significant. In animal experiments and in the Michigan farmers, exposure was by ingestion of the halogenated hydrocarbons. Perhaps cutaneous contact does not cause as significant internal organ dysfunction as ingestion because of decreased absorption. The question of bioavailability of TCDD contaminated soil was recently studied by McConnell et al. (1984) and found to induce symptoms in animals fed the soil.

Future studies need to address actual TCDD exposure, perhaps by measuring TCDD in fat tissue. In addition, subjects should be examined during the exposure, as recovery of the immunotoxicity is possible. Finally, inclusion of other immune studies such as CTL responses, which may be a more sensitive assay of TCDD immunotoxicity, should be performed. Finally, are there long-term health effects of TCDD exposure, such as the development of soft tissue sarcomas? Though a number of papers have suggested an increased risk of soft tissue sarcomas in occupationally TCDD exposed individuals (Hardell and Eriksson 1981; Moses and Selikoff 1981; Cook 1981; Honchar and Halperin 1981; Hardell and Sandstrom 1979; Sarma and Jacobs 1982), this remains unproven. Further long-term epidemiological studies should address these questions.

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