In Utero and Postnatal Exposure to 2,3,7,8-TCDD in Times Beach, Missouri: 1. Immunological Effects: Lymphocyte Phenotype Frequencies

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ABSTRACT

Analysis was performed on lymphocyte phenotype frequencies in 15 children born to mothers who resided in a TCDD contaminated environment during and subsequent to pregnancy. The tests were performed on these children at the ages of 9 to 14 years and revealed significantly low frequencies of the T-helper-inducer (CD29/CD4) subset and elevated frequency of the CD8 subset. There were also decreased levels of light chain bearing cells. These results are consistent with previous analyses of exposed human populations, as well as recent *in vitro* studies of human lymphocyte phenotype frequencies and *in vivo* studies in the marmoset monkey. The results appear to demonstrate that immune defficiencies caused by *in utero* and post-natal exposure to TCDD may persist for 10 years or more.

INTRODUCTION. In 1971, 1972, and 1973 waste oil contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was sprayed on roads and a number of horse arenas in eastern Missouri. The roads of the city of Times Beach were among those sprayed. Immediate effects included the deaths of 48 of 85 exposed horses at horse farms beginning in June, 1971.[1] In November, 1982, the EPA, as part of a state-wide review of potential TCDD contaminated sites, initiated testing which eventually revealed a range of TCDD contamination from non-detectable to 1800 ppb in soils around the roadsides of Times Beach. Studies undertaken ten years after the evacuation of Times Beach further revealed that TCDD could be found in dust in the second floors of homes in a range of concentrations from 0.026 to 2.2 ppb.

Previous studies of Missouri populations potentially exposed to TCDD included a pilot epidemiological study which examined health effects among 800 residents of the affected communities, originally chosen by means of a questionnaire.[2] The 130 selected displayed no statistically significant differences, though a number of trends, including a lower CD4:CD8 T-lymphocyte ratio, were reported. A second immunological evaluation was made of the tests of the residents of the Quail Run Trailer Park, including a number of children.[3] The study concluded that TCDD exposure in humans significantly decreased cell mediated immunity and the proportion of circulating T-lymphocytes. A further study seeking clinical correlates with body fat

TCDD burdens in 41 people with exposure histories displayed, among other findings, significant increases in both the percentage and absolute number of CD8 T-cells and a non-significant decrease in the CD4/CD8 ratio. However, in none of these studies were lymphocyte proliferative frequencies of children as a group noted.[4]

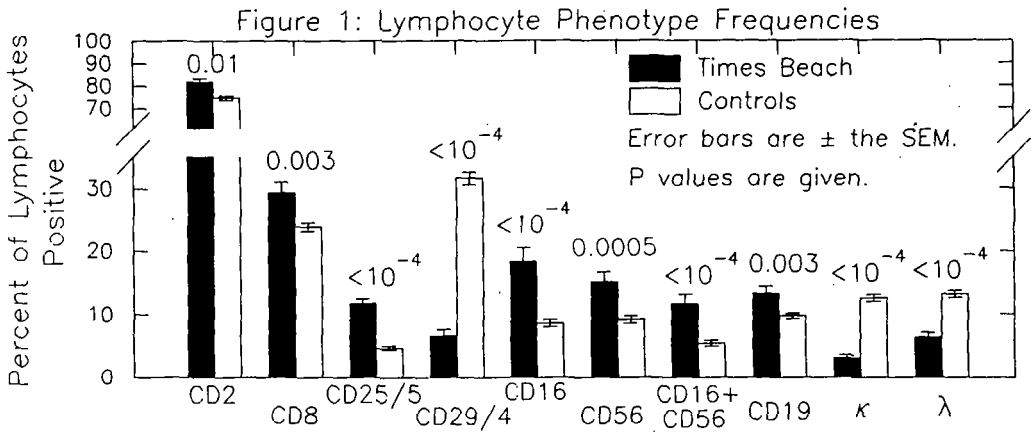
In light of a concluding comment by Webb *et al.* in 1989[4] stating " ... it is important to define and evaluate ... susceptible groups such as children who were exposed during development of their immune system," this study was undertaken. Reported here are peripheral lymphocyte phenotypes of fifteen children born between 1977 and 1983 whose parents made them available for several days of extensive testing and whose mothers resided in Times Beach during and subsequent to pregnancy while the town was contaminated with TCDD. Times Beach was evacuated in 1983, the residents' homes being bought by the Federal Emergency Management Agency. After that time the children would be unlikely to have had exposures in excess of those of the rest of the U. S. population. The children were between the ages of 9 and 14 years at the time of testing.

MATERIALS AND METHODS. Lymphocyte phenotype frequency analysis was performed on 0.1 ml aliquots of Na_3EDTA anticoagulated whole blood in disposable glass tubes. Two color combinations of fluorescein isothiocyanate (F) or phycoerythrin (PE) labelled monoclonal antibodies were added at manufacturer's recommended concentrations. Staining was for 30 min in the dark at 4 C. The tube contents were washed in 3 ml of phosphate buffered saline (PBS; 0.1M, pH 7.4, 0.1% NaN_3) by centrifugation at 500xG for 3 minutes. Erythrocytes were lysed in lysing solution (Becton Dickinson) for 8 minutes at room temperature, and remaining leukocytes were washed twice in PBS and then fixed in 1% paraformaldehyde in PBS. Gentle vortex mixing was done after the addition of antibodies and after each wash step. In one situation, where the antibody was directed to immunoglobulin light chains, the whole blood aliquot was washed twice in PBS and resuspended in 0.1 ml PBS prior to antibody addition. Monoclonal antibodies from Coulter included anti-CD1, CD2, CD3, CD4, CD8, CD29, CD45RA, CD16, CD56, DR, CD10 and CD19. Isotope controls IgG1 and IgG2a labeled with F and PE were also obtained from Coulter. Anti-light chain antibodies (κ and λ) were from GenTrak. Samples were analyzed with an Epics C flow cytometer (Coulter) with biohazard flow cell and 5w laser emitting at 488 nm. Lymphocyte gating was confirmed with CD45(F)/CD14(PE) to detect monocytes included in the gate. A minimum of 2000 lymphocytes were analyzed for each sample.

Controls for the lymphocyte phenotype frequencies were developed concurently by the Immunotransplant Laboratory, Memorial Medical Center, Springfield, IL, using from 72 to 113 normal human blood samples, including 79 females 11-50 years of age and 39 males 24-67 years of age. Although mostly adult controls were used, T-cell subset maturation is believed to occur by nine years of age.[5] Group comparisons were by student t test (two tail).

RESULTS AND DISCUSSION. Results of lymphocyte phenotype frequency analysis did not detect any differences from control frequencies for the following phenotypes (phenotype,

mean % ± standard deviation for the 15 children): CD1, 0.57% ± 0.37; CD3, 69.1% ± 9.3; CD3/DR 3.3% ± 1.8; CD4 42.8% ± 7.6; CD4:CD8 ratio 1.52 ± 0.6; CD45RA/CD4, 19.2% ± 7.9; DR, 13.2% ± 5.2; CD10/CD20, 2.6% ± 1.1. There were significant differences between subjects and controls for the ten phenotypes shown in Figure 1. The frequencies of CD2 and CD8 T-cells were significantly higher in the children than in the controls. The frequency of T-cells bearing IL-2 receptors was almost 3-fold greater than the controls. The frequency of the helper-inducer T-cell (CD29/CD4) was significantly less than in controls. Both natural killer phenotype frequencies and the frequency of coexpressed CD16/CD56 were significantly greater, as was the frequency of the early B-cell phenotype CD19. Yet, the frequency of κ and λ light chain bearing B-cells (CD19) was significantly less in the children than in the controls.



TCDD is a potent immunosuppressant in laboratory animals. Effects include changes in innate and acquired immunity, including both humoral (antibody) and cell-mediated immune responses.[6] It is believed that one mode by which TCDD may act is by impairing the function of helper T-cells, leading to an impairment of B-cell activation[7] and suppression of B lymphocyte maturation.[8] While the present findings are consistent with this understanding, of greatest interest is the marked concordance of these results with a series of studies by Neubert, *et al.*[9], on the non-human primate *Callithrix jacchus* (marmoset monkey) and on human lymphocyte cell populations. In these experiments the marmosets were dosed *in vivo* and their cell populations examined *in vitro*, while the human cells from normal donors were dosed and examined *in vitro*.

Indeed, the first of the studies performed on marmosets revealed that a single subcutaneous dose of 10 ng TCDD/kg body weight resulted in a predominant effect on cells of the helper-inducer phenotype (CD4+CDw29+) with a reduction of at least 50% of such cells compared to controls. A significant increase in the percentage of CD8+ cells was also found, but in the absence of a significant effect on the percentage of CD4+CD45RA+ (suppressor-inducer) cells. A slight increase in the percentage of CD56+ (HNK-1) cells was also noted.[9]

In a subsequent comparative study on the effects of TCDD on pokeweed mitogen (PWM) stimulated proliferation and differentiation of peripheral lymphocytes from the marmoset and man, the main targets of action of TCDD were determined to be the CD4CDw29 cells.[9] This

study also noted decreases in B-cells bearing IgG κ or λ chains and a slight reduction in the number of CD2+CD25+ cells.

The results reported here indicate a marked concordance with the findings of Neubert, *et al.* on marmosets and human lymphocyte phenotypes.[9] Thus, it would appear that the effects of TCDD on humans exposed *in utero* and postnatally may be detected 9-14 years after initial exposure in terms of T-cell activation, elevated frequency of CD8 T-cells, decreased frequency of helper inducer T-cells (CD 29/4), elevated frequency of NK cells, and decreased frequency of light chain bearing B-cells in the presence of elevated levels of early B-cells (CD19).

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