VARIATION IN HUMAN LYMPHOCYTE SUBPOPULATIONS AFTER POKE WEED-INDUCED PROLIFERATION IN THE PRESENCE OF TCDD IN VITRO

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ABSTRACT

Using a mixed lymphocyte culture and stimulation of the proliferation with optimal concentrations of poke weed mitogen we tested the effect of 2,3,7,8-TCDD on various subpopulations. For analysis monoclonal antibodies and flow cytometry (FACScan) were used.

The cell number of most of the lymphocyte subfractions increased three to four-fold during the three-day incubation period. When 2,3,7,8-TCDD was present in the culture medium during incubation, the proliferation of some defined cell subpopulations was clearly decreased. The cell populations predominantly affected were the $CD4^+CDw29^+$ cells (helper-inducer T cells) and the $CD20^+$ cells (B cells). The final concentration of TCDD in the incubation medium needed for a significant inhibition of the proliferation of these cell populations was between 0.8 and 80 fM (i.e. 1×10^{-15} to 1×10^{-13} M).

This is the lowest concentration of 2,3,7,8-TCDD reported, up till now, to induce a biological effect. The results also suggest that TCDD is capable of inducing a direct effect on isolated peripheral lymphocytes, besides possible effects on lymphocyte maturation in the thymus.

KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Human lymphocytes; Poke weed mitogen

ABBREVIATIONS

TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
DMSO = Dimethylsulfoxide;
PCDDs = Polychlorinated dibenzo-*p*-dioxins;
PCDFs = Polychlorinated dibenzofurans;
PWM = Poke weed mitogen;
MAB's = Monoclonal antibodies

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been reported to induce several adverse effects on the mammalian immune system at comparatively high (and highly toxic) doses (e.g. Vos and Moore 1974; Vos et al. 1974; Vecchi et al. 1980). Recently we reported that, in a non-human primate (*Callithrix jacchus*), rather small single doses (10 ng TCDD/kg body wt) induced subtle changes in the percentage of lymphocyte subpopulations in peripheral (venous) blood *in vivo* (Neubert et al. 1990b). The subpopulations predominantly affected include the helper/inducer T cells ($CD4^+CDw29^+$) and a subset of B cells ($CD20^+$).

Since most investigators relate effects induced by TCDD on the immune system to effects on the thymus (Cook et al. 1987; Greenlee et al. 1985a; 1985b; 1987; Dencker et al. 1985), we were interested whether a direct effect on lymphocytes could also be demonstrated. For this purpose we used the technique of the mixed lymphocyte culture (T cells, B cells and monocytes), and man as a donor of the lymphocytes. It was also our intention to develop a simple and sensitive *in vitro* system for comparative studies on the biological actions of "dioxins" and similar pollutants.

MATERIAL AND METHODS

Mixed cultures of human lymphocytes
Venous blood was obtained from single, apparently healthy donors. Clotting was prevented by addition of 50 units of heparin/ml. The lymphocyte fraction was isolated from the peripheral blood using a Ficoll^{R} separation procedure (Böyum 1966).

The freshly prepared lymphocytes were suspended in RPMI 1640 medium (Seromed, Berlin) with $NaHCO_2$, L-glutamine, penicillin/streptomycin and 5% fetal calf serum (Seromed, Berlin). The optimal poke weed (Sigma, Deisenhofen) concentration to be used was determined for each donor. From this lymphocyte suspension 0.1 ml were used together with 0.1 ml of the RMPI 1640 medium described for incubation (3 days) at 37°C in air with 5% CO_2. Poke weed mitogen and TCDD were present during the entire incubation period.

Measurement of the lymphocyte subpopulations with the FACScan
Before and after incubation the number of cells was measured and the percentages of the various cell subpopulations were analysed. After two washings with PBS (phosphate buffered saline; Biochrome Berlin) the cells were incubated at 4°C for 45 min with the different MABs, and the percentages of the cells were determined with the FACScan (Becton Dickinson). The MABs were obtained from Coulter Electronics (Krefeld, FRG) or from Becton Dickinson (Heidelberg, FRG).

2,3,7,8-TCDD was purchased from Cambridge Isotope Laboratories (Woburn, USA). Stock solutions as well as dilutions were made in DMSO, and from these solutions 0.01 ml were added to 50 ml culture medium. Thus, the assay contained a final concentration of 0.02% DMSO. A three-fold higher concentration of DMSO did not exhibit any influence on the lymphocyte proliferation.

Statistical evaluation
For statistical evaluations we used a standard software program (Minitab, Pennsylvania State College, 1987).

RESULTS AND DISCUSSION

These studies were performed with lymphocytes from the blood of several donors. Very similar results were obtained in the different experimental series, although some differences in the susceptibility of the lymphocytes of different donors were noted.

The results of a typical experiment are given in the Figures 1 and 2. A clear-cut inhibitory effect on the proliferation of some of the subpopulations is obvious. This effect was seen when the percentage of the individual cell subpopulations was measured, but more informative is the analysis of the change in the absolute number of cells during incubation. It can be clearly seen that in the absence of TCDD the cell number increased during this 3-day incubation period in virtually all the subpopulations indicated. When the incubation was performed in the presence of TCDD, especially the proliferation of two subpopulations was clearly reduced: the $CD4^+CDw29^+$ cells and the $CD20^+$ cells. On the other hand, it is interesting that the cells with the CD2 epitope as well as the $CD8^+$ and the $CD4^+CD45R^+$ cells and proliferated well in the presence of TCDD. Similarly the number of monocytes was not clearly altered during incubation and by the presence of TCDD.

This result indicates that TCDD depresses only the proliferation of rather well-defined lymphocyte subpopulations during the stimulation by poke weed mitogen. The overall number of cells or the overall increase of cells was not or barely affected under these experimental conditions.

In the mixed lymphocyte culture system used extensive interactions between the cells are expected to occur (e.g. Rode and Gordon 1970; Schmidtke and Hatfield 1976; Kneightley et al. 1976): monocytes are required to activate T cells and these will induce ($CD4^+$) or suppress ($CD8^+$) the proliferation of B cells. Thus, the effect seen on the B cells may be the result of an interference of TCDD with the proliferation of certain T cell subpopulations. However, a direct effect of TCDD on both types of cells cannot be excluded from the data available. We are presently engaged in clarifying the point of attack in our lymphocyte system.

Since the system used (PWM induction of porliferation *in vitro*) is a rather "arteficial" system, not even speculations are possible on the possible medical significance (if any) of the effects observed. Since similar effects have been observed with the lymphocytes of a non-human primate, *Callithrix jacchus* (Neubert et al. 1989; 1990a) it is feasible to assess which concentrations of TCDD may occur under *in vivo* conditions in mammalian lymphocytes.

Our data clearly show that TCDD is capable of inhibiting the poke weed mitogen-induced proliferation of lymphocytes *in vitro*. Thus, we have provided evidence for a direct action of this substance on human lymphocytes. It is noteworthy that the effect may be induced with extremely small concentrations of TCDD, namely 1×10^{-15} to 1×10^{-13} M. To our knowledge this is the lowest concentration of TCDD ever reported to induce a biological effect. For this reason the system used will provide an excellent model for studying the mode of action of extremely small concentrations of TCDD and similar chemicals.

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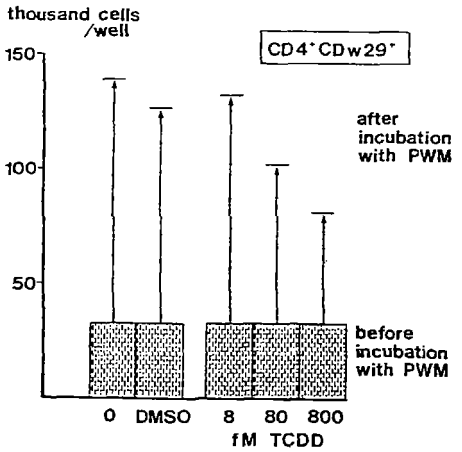

Figure 1:
Effect of various concentrations of 2,3,7,8-TCDD on the poke weed-induced proliferation of human peripheral lymphocytes *in vitro*. The culture was initiated with 1 x 10^5 cells, both poke weed mitogen and TCDD were present during the entire 3-day incubation period. Example of the changes in the number of $CD4^+CDw29^+$ cells.

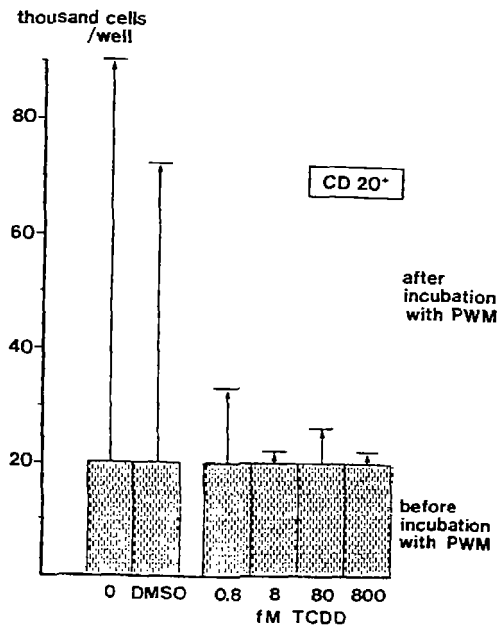

Figure 2:
Experimental conditions as in Figure 1. Example of the changes in the number of $CD20^+$ cells.