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Suppression of B Cell Differentiation by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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SUMMARY

Acute exposure of adult mice to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) results in a selective suppression of proliferating cells of the immune system, including hematopoietic stem cells and B cells. Suppression of B cell-mediated or humoral immunity, in turn, results in altered host resistance to the parasite *Plasmodium yoelii*, a malaria model. Data presented in this study demonstrate a direct effect of TCDD on cultured lymphoctes resulting in a selective inhibition of the differentiation of B cells into antibody-secreting cells. A structure-activity study suggested that this inhibition was mediated by the Ah receptor. As previously defined by receptor binding studies in hepatic cytosol, active congeners were inhibitory, whereas inactive congeners were without effect. Using lymphocytes from congenic mice which differ only at the *Ah* locus, it was determined that the Ah^{bb}-derived cells were inhibited by TCDD *in vitro*, whereas the Ah^{dd}-derived cells were not. B cell differentiation thus provides a valuable model for understanding TCDD toxicity as well as the role of the Ah receptor in growth and differentiation.

TCDD is a prototype chemical for the halogenated aromatic hydrocarbons, an important class of environmental contaminants. In spite of wide variations in species sensitivity and responses to TCDD, there are common manifestations of toxicity, as reviewed recently by Poland and Knutson (1). These include wasting syndrome, thymic atrophy, hepatomegaly, chloracne, gastric lesions, and pancytopenia. The best studied response to TCDD is induction of microsomal enzymes, especially AHH, which is involved in metabolic activation of many carcinogens. Although AHH does not appear to be directly involved in toxicity, both toxicity and enzyme induction depend upon the presence of the Ah receptor, a cytosolic receptor which binds TCDD with high affinity [see reviews by Eisen *et al.* (2) and Nebert *et al.* (3)]. Although the endogenous function of the Ah receptor is unknown, it is assumed to be critical to life processes by virtue of its presence in all species thus far examined (4).

One of the common manifestations of TCDD toxicity in rodents following perinatal exposure is immunosuppression, characterized by thymic atrophy, suppressed cell-mediated immunity, and increased susceptibility to infections (5). In contrast, acute exposure of adult mice to TCDD is characterized by a selective effect on B cell-mediated humoral immunity and a relative lack of effect on T cell-mediated immunity. For example, a single dose of 1.2 μg/kg of TCDD suppressed the primary antibody response to SRBCs for up to 42 days in Ah-responsive C57BL/6 mice, whereas lymphoproliferative responses and the ability to mediate graft-versus-host reactions were not affected (6). Suppression of the humoral immune response was subsequently shown to correlate with the susceptibility of the strain of mouse to induction of AHH using C57BL6, DBA/2, B6D2F1, and backcrosses (7). More recently, inhibition of bone marrow stem cell colony growth both *in vivo* and *in vitro* by TCDD was shown to correlate with *Ah* responsiveness in BCF, DBA/2, and the B6D2F1/J × DBA/2 backcross (8). Thus, immunosuppression by TCDD appears to segregate with the *Ah* locus in certain mouse strains as do other forms of toxicity. In fact, many environmental chemicals which are AHH inducers have been demonstrated to be immunosuppressive in the mouse. Suppression of the PFC response by polychlorinated biphenyls depends upon the ability of the isomer used to interact with Ah receptor as well as on the *Ah* responsiveness of the mouse strain tested (9). Methylcholanthrene-mediated immunosuppression in mice also segregates with the *Ah* locus (10). These chemicals require much higher doses than TCDD for AHH induction and immunosuppression, presumably due to their more rapid metabolism as well as to a lesser affinity for the cytosolic receptor. Nevertheless, it is clear that immunotoxicity of many polyhalogenated and polycyclic aromatic hydrocarbons correlates with Ah receptor binding.

The present report compares the effect of TCDD on humoral immunity *in vivo* and *in vitro* using BCF splenic lymphocytes

ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHH, aryl hydrocarbon hydroxylase; ATX, adult thymectomized; BCF, C57BL6 × C3H/HeN (F1); DCDD, 2,8-dichlorobenzodioxin; DDH, diacetyldiaminohexane; HCBP, hexachlorobiphenyl; HU, hydroxyurea; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LPS, lipopolysaccharide; OCDD, octachlorodibenzodioxin; ODC, ornithine decarboxylase; PCDD, 1,2,3,7,8-pentachlorodibenzodioxin; PFC, plaque-forming cell; SRBCs, sheep erythrocytes; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TNP, trinitrobenzene sulfonic acid.

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and describes the consequence of humoral immunosuppression in terms of altered resistance to infection. The direct effect of TCDD on cultured murine lymphocytes resulted in an inhibition of the differentiation of B lymphocytes to antibody-producing cells, which was further shown to correlate with Ah receptor levels by structure-activity studies and a comparison of congenic mice differing only at the *Ah* locus. *In vitro* B cell differentiation is discussed as a model for studying TCDD toxicity at the cellular and molecular level.

Materials and Methods

Mice. Female BCF (C57BL6/N × C3H/HeN) mice, 6–8 weeks of age and weighing 18–21 g, were obtained under the National Cancer Institute production contracts from Charles River Breeding Laboratories, Portage, MI. ATX BCF mice, also from Charles River, were used 4 weeks after surgical thymectomy. Congenic mice, B6.D2-Ah^{dd} (NE9), were bred at Research Triangle Institute by Dr. Susan Lewis for Dr. Linda Birnbaum of National Institute of Environmental Health Sciences. This strain was derived from B6N.D2N-Ah^{d} (NE 13) from Dr. Daniel Nebert, then bred into C57BL/6J by backcross-intercross in the laboratory of Dr. Alan Poland. They have been genetically verified by Dr. Lewis using more than 30 biochemical markers, and offspring were periodically phenotyped by zoxazolamine paralysis. C57BL/6J mice from Jackson Laboratories (Bar Harbor, ME) were housed at Research Triangle Institute for 2 weeks and served as Ah-responsive controls for the B6.D2-Ah^{dd} mice. All animals were maintained on a 12-hr light/dark cycle at 68–73°F and were provided autoclaved food and water *ad libitum*.

Chemicals and exposure regimens. Dibenzo-*p*-dioxins, 2,3,7,8-tetrachlorodibenzofuran, and 2,4,5,2′,4′,5′-HCBP, were kindly provided by Dr. James D. McKinney, Ms. Martha Harris, and Dr. Linda Birnbaum from this institute. Qualitative and quantitative confirmation of the TCDD stock solution was provided by Mr. Donald Harvan, also of this institute, using a VG-Micromass ZAB-2F high resolution mass spectrometer with Varian 1400 gas chromatograph and Finnigan INCOS 2300 data system. For *in vivo* exposure, the TCDD was initially dissolved in a small volume of reagent grade acetone and diluted in corn oil. This was heated with stirring to allow the acetone to evaporate. Mice were administered a single dose by gavage in a volume of 0.2 ml while controls received an equal volume of corn oil. All dosing and animal maintenance were performed in a high hazard area designed to prevent exposure of personnel to animal wastes. For *in vitro* studies, TCDD was added to human AB serum (Irvine Scientific, Irvine, CA) by dissolving a mixture of unlabeled and ^{14}C-labeled TCDD (U.L. from KOR Isotopes, Cambridge, MA) in benzene such that the final specific activity was 1 μCi/μmol. The solution was added to a beaker containing glass homogenizing beads, and the benzene was evaporated under nitrogen. Serum was then poured into the beaker and allowed to incubate for 24 hr at 4° with occasional swirling. The serum was then decanted and filter-sterilized, and the concentration of TCDD was calculated, based upon the known specific activity. The preparation used in the studies described here contained 2 μM TCDD. Serum containing TCDD or matching control serum was added to the culture medium to achieve the final concentration of TCDD desired. In structure-activity studies, where radiolabeled compounds were not available, the test material was dissolved in *p*-dioxane and added directly to serum so that the final concentration of dioxane did not exceed 0.01% in the cultures.

***In vivo* antibody responses.** Mice were immunized with the T-dependent antigen, SRBCs, by intraperitoneal injection of 0.2 ml of 10% SRBCs 3 days after dosing. Four days later the spleens were assayed for PFCs using a hemolysis in agar method as previously described (11). Antibody response to the T-independent antigen LPS (*Escherichia coli* 0127 from Difco) was determined 4 days following intravenous injection of 10 μg. The coupling method of Veit and Michael (12) was used to bind LPS to SRBCs for this assay.

***In vitro* antibody responses.** Splenic cells were immunized with SRBCs *in vitro* by slight modification of the Mishell-Dutton method (13). Cells were cultured in Roswell Park Memorial Institute Medium 1640 (Gibco) containing 25 mM Hepes, 2 mM L-glutamine, 50 μg/ml of gentamycin, 5% human AB serum, and 5% fetal calf serum. Cultures consisted of 15 × 10^6 cells contained in 1 ml in 35-mm Petri plates (Falcon). Following addition of 25 μl of 1% SRBCs, the cultures were incubated at 37° with gentle rocking in an atmosphere of 7% O_2, 10% CO_2, 83% N_2. On the fifth day, cultures were assayed for PFCs and total nucleated cells. Viabilities were determined by the pronase method as previously described (14). This method is more sensitive than other viability tests such as trypan blue exclusion.[1] For polyclonal antibody production, 5 × 10^6 cells in 1 ml were cultured with 10 μg of LPS in 24-well culture plates. These cultures were stationary in a 5% CO_2 incubator. PFCs were enumerated on the third day of culture using TNP-coupled SRBCs (15).

***Plasmodium* infection.** *Plasmodium yoelii* 17XNL, a nonlethal strain of malaria, was originally obtained from Mr. Charles Evans (National Institute of Allergy and Infectious Diseases, Bethesda, MD). Organisms were maintained as a frozen stock in liquid nitrogen and passed through mice once prior to infection of experimental animals by intravenous injection of 10^6 parasitized erythrocytes. Diff-Quick-stained thin films of tail blood were performed on the indicated days following exposure, and the percentage of parasitized erythrocytes was determined.

ODC assay. The activity of ODC in lymphocyte sonicates was determined by measuring the liberation of $^{14}CO_2$ from L-[1-^{14}C]ornithine according to the method of Watanabe *et al.* (16). DL-[1-^{14}C] Ornithine (58 mCi/mmol) was purchased from New England Nuclear. Each 0.5-ml assay contained 0.5 μCi of [^{14}C]ornithine and 0.04 mM DL-ornithine and was allowed to incubate 60 min at 37° with gentle shaking. Following acidification, samples were incubated 30 min longer to ensure release of CO_2.

Statistical analysis. Data were analyzed by one-way analysis of variance followed by Dunnett's test for multicomparison with a control.

Results

The effect of a single oral dose of TCDD on the T-cell-dependent (SRBC) and T-cell-independent (LPS) antibody responses in Ah-responsive BCF mice is shown in Fig. 1A. Splenic cellularity was not affected, but the number of anti-

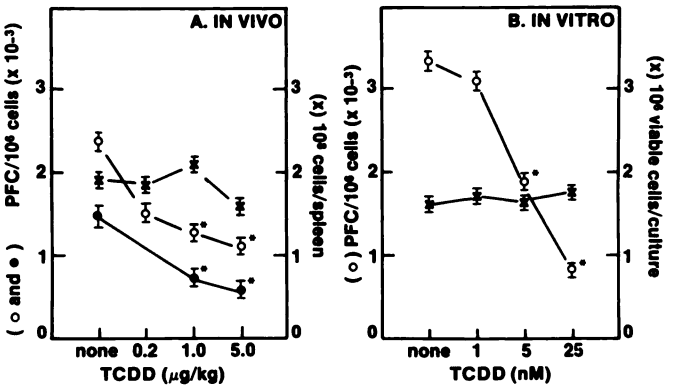

Fig. 1. Comparison of *in vivo* and *in vitro* effects of TCDD on the PFC response. For the *in vivo* study illustrated in A, TCDD was administered to five BCF female mice per group by gavage in corn oil; mice were immunized with SRBCs (○) or LPS (●) 3 days later and PFCs were determined 4 days after immunization. One μg of TCDD is equivalent to 3 nmol. For the *in vitro* study illustrated in B, TCDD was delivered to cultured splenic lymphocytes in human AB serum. The PFC response to SRBCs was determined on the fifth day of culture using four replicates per concentration. *, values which differ from control at $p < 0.05$.

[1] M. P. Holsapple, unpublished observations.

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body-producing cells, measured as PFCs, following immunization with either SRBCs or LPS was reduced at all doses of TCDD, although significantly only at 1 and 5 μg/kg. Considering that thymic atrophy is a dominant feature of TCDD toxicity, it should be emphasized that suppression of humoral immunity occurred at doses below those which caused thymic atrophy. Thymic atrophy did not occur in BCF mice at doses below 5 μg/kg (data not shown). Lack of direct thymic involvement in the suppression of humoral immunity by TCDD is also demonstrated in Table 1, which compares the response to TCDD in ATX BCF mice to sham controls. The T cell-dependent antibody response in ATX mice was suppressed 81% at 5 μg/kg of TCDD, as compared to 83% suppression in sham controls. Thymectomized mice receiving corn oil had an unimpaired T-dependent antibody response in accord with the observation that adult thymectomy does not affect antibody production in mice for up to 1 year (17).

The consequence of immunosuppression by a single dose of TCDD on host resistance is illustrated in Fig. 2. Two days after the indicated dose of TCDD, mice were infected with the nonlethal parasite, *P. yoelii*, and the course of infection was followed. The peak parasitemia, which normally occurs on day 12, was greater and of longer duration in TCDD-treated mice than in controls, the difference being significant at 5 μg/kg on day 10 and at 10 μg/kg on days 12 and 14. Humoral immunity is thought to play a major role in host resistance to malaria parasites (18).

The ability of TCDD to act directly on the lymphocyte was further demonstrated by *in vitro* immunization of splenic cells. As shown in Fig. 1B, cells cultured in the presence of TCDD exhibited a dose-related suppression of the PFC response to SRBCs with an ED_{50} of 7 nM. Viability of the cells was not affected, indicating a selective effect on the development of antibody-secreting cells. Development of PFCs was also inhibited when splenic cells were incubated for 1 hr with TCDD, collected by centrifugation, and cultured in fresh medium with antigen (Fig. 3). As is also shown in this figure, TCDD must be present early in culture for immunosuppression to result; addition as late as day 2 of culture caused little inhibition, nor was it inhibitory if present during the PFC assay on the fifth day of culture.

In vitro PFC suppression was found to correlate with the ability of a given chemical to bind to the *Ah* receptor, as determined previously in mouse liver binding studies (19). As shown in Fig. 4, several active congeners, including PCDD, TCDF, and 2,3,7-TCDD, had immunosuppressive activities similar to that of TCDD, whereas the inactive congeners, OCDD, 2,4,5,2′,4′,5′-HCBP, and DCDD, were without effect at concentrations up to 5×10^{-8} M.

The suppression of PFC development *in vitro* by TCDD was shown to depend upon the Ah genotype of the mouse by the experiment described in Table 2, in which responsive C57BL6/J (Ah^{bb}) mice were compared to nonresponsive B6.D2 mice (Ah^{dd}). Using lymphocytes from the Ah^{bb} mice, significant and dose-related suppression by TCDD was observed, which was not the case for the congenic Ah^{dd} mice. This experiment confirmed an earlier preliminary study using these strains.

We attempted to examine the possible antiproliferative effect of TCDD on cultured lymphocytes by measuring ODC activity and DNA synthesis on their respective peak days following stimulation with LPS. As shown in Table 3, ODC was stimulated by LPS but was not inhibited by TCDD. DDH was used as a positive control and caused marked inhibition of ODC at 1 mM. DNA synthesis was slightly but significantly inhibited

TABLE 1

Effect of TCDD on humoral immunity in ATX mice

Female BCF mice, five per group, were given corn oil or TCDD at 5 μg/kg orally 5 days prior to immunization with sheep erythrocytes. Splenic PFCs were determined 4 days later and are expressed as mean ± SE.

Mouse	Treatment	PFC/10^6 cells
Sham	Corn oil	1810 ± 142
Sham	TCDD, 5 μg/kg	315 ± 58*
ATX	Corn oil	1950 ± 159
ATX	TCDD, 5 μg/kg	372 ± 80*

* Significant at $p < 0.05$.

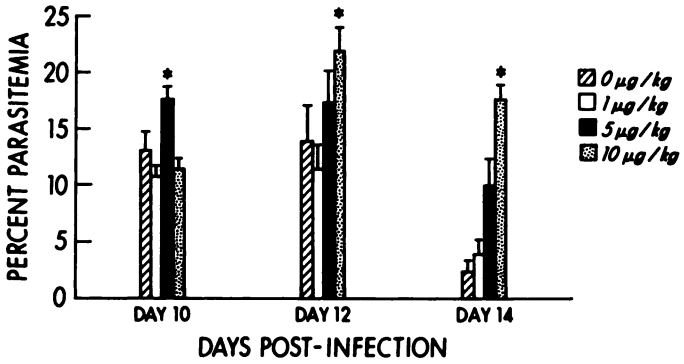

Fig. 2. Effect of TCDD on resistance of BCF mice to the parasite, *P. yoelii*. Using eight mice per treatment group, the percentage with parasitemia was determined 10, 12, and 14 days after infection and is expressed as the mean ± SE on the *bar graph*. *, significant at $p < 0.05$.

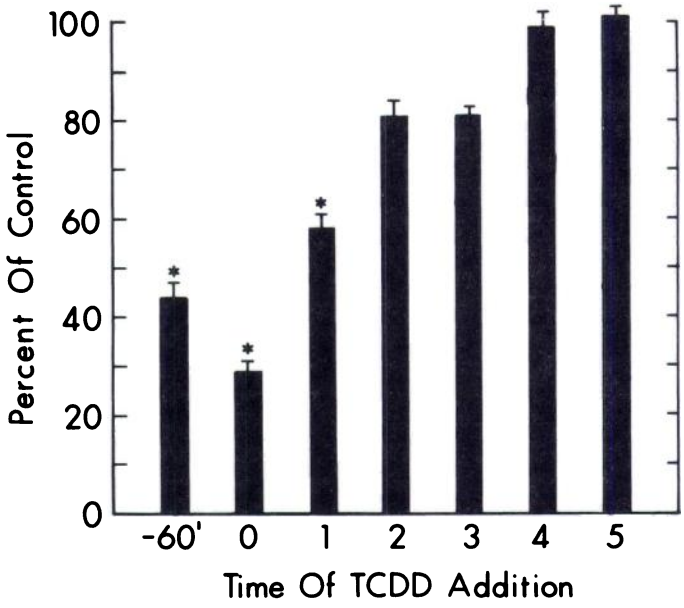

Fig. 3. Relationship between time of TCDD addition and *in vitro* PFC response. TCDD was added to splenic cells at 2×10^{-8} M on the indicated days, day 0 being the beginning of the culture period and time of antigen (SRBC) addition. The time indicated by −60′ refers to cells which were preincubated with TCDD for 60 min and washed prior to culture with antigen. The day 5 treatment was performed by adding TCDD to the cells during the PFC assay. All values are expressed as percentage of control. The control value for the preincubation addition was 13,480 ± 1,748 PFCs/culture, and background (no SRBCs) was 960 ± 98 PFCs/culture. *, values which are significantly different from control at $p < 0.05$.

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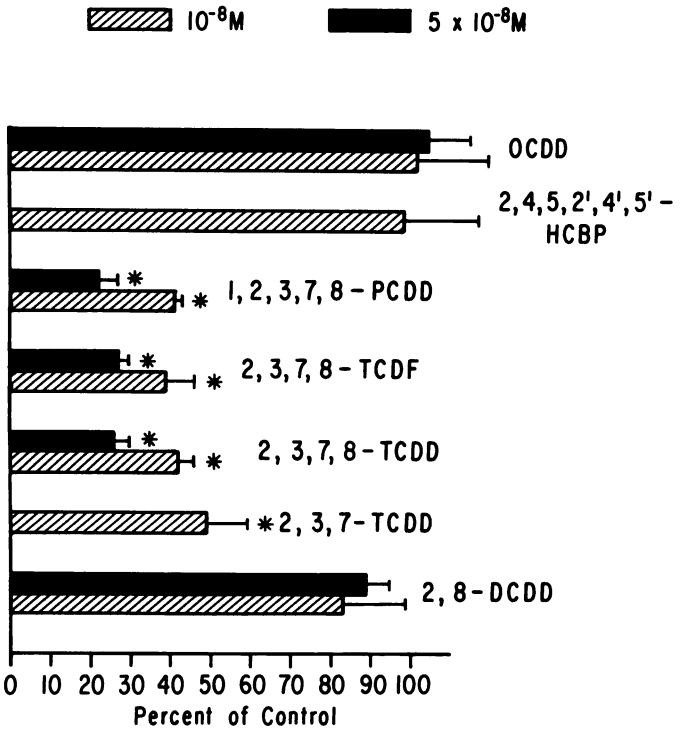

Fig. 4. Structure-activity study using *in vitro* immunized cultures. Pooled splenic cells were cultured with sheep erythrocytes *in vitro* in the presence of the indicated chemicals. TCDF is 2,3,7,8-tetrachlorodibenzofuran; 2,3,7-TCDD is 2,3,7-trichlorodibenzodioxin; Percentage of control was calculated using mean PFCs/culture of treated groups divided by mean PFCs/culture in controls. *, values which are significantly different from control at $p < 0.05$.

TABLE 2

Comparison of TCDD effect on *in vitro* PFC response in congenic mice PFCs per 10^6 cells

The PFC response to SRBCs was determined on the fifth day of culture using splenic lymphocytes from congenic mice which differ only at the *Ah* locus. TCDD was added in human AB serum to achieve the concentrations indicated.

TCDD	Ah^{bb}	Ah^{dd}
Control	4280 ± 305	4250 ± 314
2×10^{-9} M	3380 ± 232[a]	4040 ± 453
1×10^{-8} M	3090 ± 336[a]	3980 ± 366
5×10^{-8} M	2420 ± 124[a]	4180 ± 541

[a] Value differs from control at $p < 0.05$.

by TCDD and markedly inhibited by DDH and HU. The PFC response was inhibited by TCDD and HU, whereas cell yield was affected only by HU.

Discussion

The present report describes the suppression of both the T cell-dependent and T cell-independent antibody responses following antigenic challenge in adult Ah-responsive mice exposed to a single dose of TCDD by the oral route. The suppression of humoral immunity resulted in increased susceptibility of the host to the malaria parasite, *P. yoelii*. We have recently shown that diphenylhydantoin, which selectively depresses humoral immunity, also impairs host resistance to this parasite (20), further supporting the idea that humoral immunity is a major aspect of resistance to malaria (18).

The suppression of the humoral response by TCDD is clearly thymic independent since it occurred at doses below those producing thymic atrophy, in thymectomized mice, and in cultured cells. The lack of thymic effects must be emphasized since thymic atrophy is considered a hallmark of TCDD toxicity, and some aspects of TCDD-induced immunosuppression are a consequence of alterations in thymic epithelial function (21).

Based on the present observation that suppression of the antibody response to the T cell-independent antigen, LPS, and the previous report (6) that suppression of the response to the T cell-independent antigen, type III pneumococcal polysaccharide, occurred in the same dose range as the suppression of the T cell-dependent response to SRBCs, the effect of TCDD would appear to be on the B cell. Hexachlorodibenzo-p-dioxin (22) and several polycyclic aromatic hydrocarbons which are AHH inducers have also been shown to have a selective effect on the B cell (23, 24). Although T cell regulation cannot be totally ruled out, the humoral immunosuppression by TCDD clearly occurs at the cellular level as evidenced by the inhibition of PFCs following *in vitro* exposure of splenic lymphocytes to low concentrations of TCDD. Addition of TCDD to cultured lymphocytes resulted in a selective suppression of PFCs following stimulation with SRBCs or LPS, whereas cellular viability was not affected. These data suggest that differentiation, rather than proliferation, is affected by TCDD, possibly as a consequence of impaired interaction of differentiation factors with B cell membrane receptors or down regulation of these receptors. In order to better address this question, we measured DNA synthesis and ODC activity in LPS-stimulated lymphocytes. ODC, which has been shown to be preferentially associated with proliferation in leukemia cells (25), was not inhibited by TCDD under the conditions used (Table 3). There was a significant suppression of DNA synthesis when measured on peak day (day 2 of culture). This difference was not observed

TABLE 3

Comparison of TCDD effects on proliferation and differentiation of B cells stimulated with LPS

Splenic cells from BCF mice were cultured with LPS and other additions, as indicated. ODC activity was measured on day 1 and is expressed as pmol/mg of protein. DNA synthesis was measured on day 2 by ^{3}H-Tdr incorporation by liquid scintillation spectrometry of filtered, washed cells following a 6-hr pulse with 1 μCi/ml of culture and is expressed as 10^3 cpm/culture. PFCs were measured on day 3 using TNP-SRBCs and are expressed as PFCs/culture. The cell recovery on day 3 is also given (10^6 cells). All values are means ± SE of four cultures.

Inhibitor	ODC	DNA	PFCs	10^6 Cells
No LPS	3 ± 0.4	2.1 ± 0.2[a]	15 ± 6[a]	3.0 ± 0.2[a]
Control	116 ± 1.3	43.2 ± 0.7	1730 ± 38	4.5 ± 0.1
10^{-8} M TCDD	149 ± 31	34.5 ± 0.7[a]	1070 ± 43[a]	4.6 ± 0.2
2×10^{-8} M TCDD	128 ± 28	33.2 ± 0.6[a]	605 ± 21[a]	4.5 ± 0.1
10^{-3} M DDH	32 ± 4[a]	13.1 ± 0.2[a]	1520 ± 29	4.1 ± 0.1
10^{-4} M HU	ND[b]	20.7 ± 0.4[a]	714 ± 35[a]	2.8 ± 0.1[a]

[a] Value significantly different from control at $p < 0.05$.
[b] ND, not done.

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on day 3 (data not shown) when PFCs were enumerated. It is difficult to say at this point what the biological consequences of such a mild suppression of DNA synthesis might be. Proliferation is difficult to separate from differentiation since differentiation requires a commitment to one round of cell division (26). Clearly, further studies concerning cell cycle analysis are warranted. Standard lymphoproliferative responses to B and T cell mitogens are not affected by TCDD *in vivo* (6) or *in vitro* (data not shown).

Other cells of lymphoid origin appear to be unresponsive to maturational signals in the presence of TCDD *in vitro* as well as *in vivo*. For example, granulocyte-macrophage progenitor cells from bone marrow (CFU-GM) are very sensitive to TCDD *in vitro*, exhibiting inhibition of growth in response to colony-stimulating factor at concentrations of TCDD as low as 10^{-10} M (8). Cell surface receptors for epidermal growth factor have been shown to be reduced as a result of exposure of fibroblasts or keratinocytes to AHH inducers (27, 28), suggesting that some membrane receptors are down regulated by compounds which bind to the Ah receptor. In the case of the lymphocytes, it is important to realize that TCDD must be added early in the culture period, prior to day 2, in order to inhibit the development of PFCs. This may imply that there is a necessary time for altered genetic expression to occur or that the critical event is cell cycle dependent. Most of the events involving expression of receptors for growth and differentiation factors would be expected to occur very early in the culture period followed by a period of several days necessary for committed cells to differentiate into antibody-producing cells. In this respect, B cell differentiation represents a valuable model for studying TCDD toxicity at the molecular level since the factors and events involved are beginning to be understood (26).

The structure-activity study provides further evidence for the correlation of Ah receptor occupation with immunosuppression, since four active compounds produced significant suppression of the *in vitro* PFC response and three inactive compounds were without effect (Fig. 3). The inactive dibenzodioxin congeners were identified by their lack of binding in the Ah receptor assay (19), and the HCBP isomer used has been shown to be a poor inducer of AHH activity in rat liver (29). Although indirect, this evidence clearly implicates binding of TCDD to the Ah receptor of the lymphocyte as being an essential event in immunosuppression. Previous genetic studies (7) and the data described in Table 2, comparing the sensitivities of lymphocytes from congenic mice differing only at the *Ah* locus to TCDD *in vitro*, further implicate the Ah receptor in immunosuppression. Studies are under way in this laboratory to better define the interaction of TCDD with the lymphoid cells by measuring the Ah receptor levels in the various cell populations involved, including the B lymphocyte. Receptor levels have recently been quantified on bone marrow stem cells in BCF and DBA mice, and these levels correlate with TCDD myelotoxicity (8). Induction of AHH in both human (30) and murine (31) lymphocytes has been reported, further suggesting the presence of a specific receptor on these cells. More importantly, in the case of the human lymphocytes, AHH was induced by TCDD in cultured lymphocytes at 7×10^{-9} M, which is in the concentration range that resulted in inhibition of the PFC response in the present studies. Cultured lymhocytes thus provide a system in which one can examine the relationship of enzyme induction to toxicity, and these studies are now under way in this laboratory.

The endogenous function of the Ah receptor is the subject of intense investigation since it is presumed to play a critical role in cellular differentiation. Hepatic cytosolic Ah receptors from all species thus far examined exhibit remarkable homology and similar amounts of receptor with the exception of the DBA/2 mouse. The tissue distribution is also very similar between species with the exception of the hamster (4). Although the interaction of TCDD with the Ah receptor is required, it does not appear to be sufficient for toxicity (1). In view of the wide differences in species and tissue responses to TCDD, it is clear that additional factors are involved. It is thus important to more fully characterize the spectrum of TCDD effects in more tissues which appear to be sensitive "target tissues," such as the lymphocyte. Models such as the one described here concerning B cell differentiation may help to understand TCDD toxicity and, in turn, elucidate the endogenous role of the Ah receptor in growth and differentiation. In this respect, the B cell model is especially useful as lymphocytes express several structurally characterized gene products at their surface which are involved in specific proliferation and differentiation processes.

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