0026-895X/89/010018-08$02.00/0

MOLECULAR PHARMACOLOGY, 35:18-25

Lymphocyte Stem Cell Alterations following Perinatal Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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Received July 22, 1988; Accepted September 23, 1988

SUMMARY

Perinatal exposure of experimental animals to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) leads to thymic atrophy and a suppression of cell-mediated immunity that is more severe and persistent than that caused by adult exposure, suggesting that events involved in the maturation of the immune system are particularly sensitive to TCDD. We report here that perinatal TCDD exposure produces an alteration in the lymphocyte stem cell population in the fetus and neonate, as evidenced by a significant reduction in the lymphocyte stem cell-specific enzyme terminal deoxynucleotidyl transferase (TdT). After maternal treatment with a single dose of TCDD (10 μg/kg of body weight) on gestational day (gd) 14, TdT biosynthesis and TdT-specific mRNA were reduced more than 50% in fetal liver lymphoid cells on gd 18. An even more extensive reduction was seen in neonatal bone marrow through postnatal day 18. In contrast, thymic TdT synthesis appeared to be relatively unaffected on a per cell basis by perinatal TCDD exposure, although the actual number of TdT-synthesizing thymocytes was diminished due to extensive thymic atrophy. These effects occurred at concentrations of 1–31 fg of TCDD/mg of thymus. Flow cytometric analysis of thymocyte surface marker expression revealed a slight decrease in the percentage of Lyt-2$^+$L3T4$^+$ thymocytes on gd 18 and postnatal day 4. This alteration was no longer apparent by postnatal day 11, when marrow TdT biosynthesis was most suppressed. These results suggest that TCDD-induced thymic atrophy during the perinatal period may be due, in part, to an effect on the prothymocyte.

The thymus is seeded by lymphocyte stem cells originating postnatally from bone marrow and in the fetus from the liver and yolk sac (1). These cells, termed prothymocytes, are believed to be committed to the T lymphocyte lineage yet do not express the characteristic T cell surface markers found in the thymus (2, 3). Within the thymus, these cells undergo a series of differentiation and selection events, which result in the generation of a diverse, functional, T lymphocyte repertoire (4). Evidence suggests that at least two distinct, although developmentally related, subsets of prothymocytes exist, one that contains the enzyme TdT and one that does not (5, 6). TdT is also produced by immature cortical thymocytes and some subsets of pre-B cells but is not found in mature lymphocytes, other leukocytes, or CFU-S (7–10), indicating that this enzyme is restricted to lymphocyte stem cells. In both early B and T lymphocyte development, TdT promotes the insertion of novel genetic material (N-regions) between the sequences coding for the variable and constant regions of both immunoglobulin heavy chain and T cell receptor molecules (11, 12), implying that this enzyme is involved in the generation of immunological diversity. To date, TdT is the only reliable marker for fetal liver and bone marrow prothymocytes.

The polyhalogenated aromatic hydrocarbon compounds, of which TCDD is the most biologically potent, produce a number of toxic manifestations, all of which appear to be mediated by their binding to the *Ah* receptor and the subsequent association of the ligand-receptor complex with specific nuclear sites, resulting in altered gene expression (13). This receptor appears to serve as a gene regulatory protein and is structurally and functionally similar to the steroid hormone receptors, although its endogenous ligand has not yet been identified. The *Ah* receptor is found in thymus, bone marrow, and spleen (14–17), suggesting that it has a role in the generation and/or functionality of the hematopoietic and immune systems. Indeed, these systems appear to be extremely sensitive to perinatal TCDD

This work was supported by National Institute of Environmental Health Sciences Grants ES02515 and ES01247, an award from the International Life Sciences Research Institute Foundation to T.A.G., and Grants CA42737 and CA42128 from the National Cancer Institute to A.E.S. A.E.S. is a Scholar of the Leukemia Society of America. J.S.F. was supported by National Institute of Environmental Health Sciences Toxicology Training Grant ES07026.

ABBREVIATIONS: TdT, terminal deoxynucleotidyl transferase; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; gd, gestational day; CFU-S, colony-forming unit-spleen; *Ah*, aryl hydrocarbon; IL-2R, interleukin-2 receptor; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; DP, double positive; DN, double negative.

exposure, as evidenced by severe thymic atrophy and suppression of cell-mediated immune function (18), as well as myelotoxicity (19).

One possible cause of thymic atrophy and suppression of cell-mediated immunity may be an alteration to the lymphocyte stem cell compartment, resulting in an inhibition of lymphopoiesis. We report that perinatal TCDD exposure produces such an alteration in fetal liver and neonatal bone marrow lymphocyte stem cells, as determined by a reduction in TdT biosynthesis and mRNA levels. These alterations occur at very low TCDD levels and do not appear to be linked to any profound alterations in the relative percentage of cells in various intrathymic subpopulations.

Materials and Methods

Animals and treatment. Timed pregnant BALB/cGa mice were obtained from the Inbred Mouse Unit of the Environmental Health Sciences Center (day of vaginal plug = day 0). Animals were maintained on a 12-hr light/dark cycle and were provided food and water *ad libitum*. Unlabeled TCDD (Cambridge Laboratories, Cambridge, MA) in anisole was dissolved in olive oil and the anisole was removed under nitrogen. Pregnant mice received a single dose of TCDD in olive oil (10 μg of TCDD/kg of body weight) in a volume of 0.1–0.2 ml by gavage on gd 14. This day was chosen for dosing because it is the earliest time that avoids cleft palate induction, which would hinder suckling and hence postnatal exposure to TCDD via the milk (20). To determine tissue TCDD levels, [^{3}H]TCDD (38.0 mCi/μmol), the generous gift of Dr. Stephen Safe (Texas A&M University, College Station, TX), was prepared and administered in a similar manner. In all experiments, controls received olive oil only. For postnatal studies, litters were normalized to five pups/litter on postnatal day 2 to allow for uniform TCDD exposure via nursing. For the studies described, gd 18 fetuses and postnatal day 4, 11, and 18 pups (day of birth = postnatal day 0) were sacrificed by chilling and CO_2 overdose. Weights of individual pups were determined at the time of sacrifice. At each time point, tissues from 5–15 litters were pooled.

Cell isolation. Thymus organs from individual pups were excised, cleaned, and weighed, and the thymocytes were released by mincing the organ in cold M199 media (GIBCO, Grand Island, NY) containing 5% NU-IV serum (Collaborative Research, Lexington, MA) and triturating the tissue with a bent 18-gauge needle. Debris was eliminated by passing the cell suspension through 75-gauge nylon gauze. To obtain bone marrow cells, femurs and tibias were dissociated free of attached tissue, both ends of the bone were cut at the epiphysis, and the shafts were flushed with cold M199 plus 5% NU-IV. Enrichment for fetal liver lymphoid cells was performed by mincing gd 18 livers in this medium and passing the chunks through a nylon gauze. In each case, cells were pelleted, erythrocytes were lysed with ACK buffer (0.17 M NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA), and the cells were washed with M199 plus 5% NU-IV. Cell yield and viability were determined by light microscopy after incubation with Trypan blue dye. At each time point examined, cells from each organ were pooled for analyses.

[^{3}H]TCDD tissue levels. For radiolabel studies, liver and thymus were obtained from gd 18 mice and from neonates; spleens were removed from neonates only. Tissues were removed, cleaned, and digested overnight in NCS solubilizer (Amersham, Arlington Heights, IL) at 50° with constant swirling. One milliliter of acetic acid was added to neutralize pH, scintillation fluid was added, and each sample was counted in a Packard-TriCarb 4530 scintillation counter.

TdT immunoprecipitation assay. Thymocytes, bone marrow cells, and fetal liver lymphoid cells (2×10^7) were metabolically labeled with 250 μCi of [^{35}S]methionine in 2.0 ml of methionine-free Eagle's minimum essential medium (GIBCO) supplemented with 10% dialyzed, heat-inactivated, fetal calf serum (Hyclone, Logan, UT), 50 μM 2-mercaptoethanol, and 2 mM L-glutamine for 3 hr. Cells were washed in phosphate-buffered saline and lysed in PLB (10 mM sodium phosphate (pH 7.5), 100 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 1% sodium dodecyl sulfate). Aprotonin and phenylmethylsulfonyl fluoride were added to the lysates to minimize proteolysis (21). Samples were precleared using formalin-fixed *Staphylococcus aureus* (Pansorbin; Calbiochem, La Jolla, CA) conjugated to rabbit IgG. Precleared lysates were incubated 16–20 hr with rabbit anti-calf TdT serum (Pharmacia, Piscataway, NJ), and the antigen-antibody complexes were precipitated with Pansorbin. Dissociated and reduced complexes were run under reducing conditions on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. For fetal liver, thymus, and bone marrow, immunoprecipitates from 2×10^7, 1×10^6, and 8×10^6 cpm, respectively, were loaded per lane. Gels were dried, fixed, and autoradiographed as previously described (21). Quantification of immunoprecipitated protein was determined from the optical density of the 60-kDa band (58 kDa for fetal liver) on the autoradiogram. Experimental normalization was achieved by similar quantification of the 45 kDa actin band, which is immunoprecipitated by naturally occurring anti-actin antibodies present in normal and immune serum. Quantified autoradiograms for a particular exposure were determined to be within the linear range of the film, and optical density measurements were made with a Bio-Rad (Richmond, CA) model 620 Video Densitometer. Peak output was quantitated with a Hewlett-Packard 3392A integrator.

TdT mRNA determinations. Total cellular RNA was isolated using a guanidinium thiocyanate method (22) and quantified by optical density at 260 nm. Five micrograms and 4-fold sequentially diluted samples were adsorbed under vacuum onto nitrocellulose paper using a Bio-Rad Bio-Dot SF slot-blot apparatus. ^{32}P-labeled TdT probe was prepared from a previously described (22, 23) purified cDNA fragment, pTdT 20, and the random oligonucleotide labeling procedure (24). Hybridization was carried out as previously described (22), and autoradiograms were exposed at −70° to obtain exposures within the linear range for at least two dilutions of a particular sample. After removal of ^{32}P-labeled cDNA by boiling, the filters were hybridized with ^{32}P-end-labeled poly(dT) to quantitate total poly(A) RNA in each sample. Autoradiograms were obtained, and both TdT and poly(A) autoradiograms were quantified densitometrically as described above. TdT values were normalized to the total amount of poly(A) RNA detectable in a sample, as previously described (22).

Monoclonal antibodies. All antibodies used were titrated and used under saturating conditions. Anti-Lyt-2 (clone 53-6.7) and phycoerythrin-conjugated anti-L3T4 (clone GK1.5) were purchased from Becton-Dickinson (Sunnyvale, CA). The antibody 7D4, which identifies an epitope on murine IL-2R, was prepared from the culture supernate of a clone obtained from the American Type Culture Collection (Rockville, MD) and used at a 1:2 dilution. FITC-conjugated goat anti-rat IgG and FITC-goat anti-rat IgM were obtained from Cappel Laboratories (Malvern, PA). Suspending medium was Hank's balanced salt solution (GIBCO) containing 0.1% bovine serum albumin and 0.1% NaN_3.

Staining procedures and multiparameter flow cytometric analysis. For determination of thymocyte Lyt-2/L3T4 subpopulations, cells from control or TCDD-exposed fetuses/pups were pooled and 1–2.5 $\times 10^6$ cells from each group were incubated with anti-Lyt-2 (2.5×10^8 cells/ml) for 30 min in the dark on ice. The cell suspension was then underlayed with calf serum and centrifuged to a pellet, and the supernatant was removed. FITC-goat anti-rat IgG was added and the cells were incubated and washed as before. After a 30-min serum blocking step, an optimal concentration of phycoerythrin-conjugated-anti-L3T4 was added. Each experiment included samples in which one or the other primary antibody was omitted to insure that cross-reactivity or competition did not occur. For measurement of IL-2R surface expression, the cells were incubated with 7D4, underlayed with serum, centrifuged, and incubated with FITC-anti-rat IgM. After staining, all samples were washed once in suspending medium and the cells were resuspended in Hank's balanced salt solution with 1% paraformal-

dehyde. The samples were stored in the cold and in the dark until analysis.

Laser flow cytometric measurements were performed using an EPICS-V flow cytometer sorter (Coulter Electronics, Hialeah, FL) equipped with a UV-enhanced argon ion laser (Spectra Physics model 2025). Cells were excited using the 488-nm line at 500 mW. Fluorescence emissions from phycoerythrin and fluorescein was separated by a 560 nm dichroic mirror. A 575-nm band-pass filter and a 530-nm band-pass filter were used to separate phycoerythrin and fluorescein emissions, respectively. Electronic spectral compensation was performed using the subtraction module. For each sample, 30,000 cells were analyzed; cells were gated based on light-scatter-pulse-width-time of flight, a measure of cell diameter (using a module designed and constructed by J. Leary, University of Rochester Medical Center) and peak low angle light scatter (via a modification designed by J. Leary). All data were analyzed on a 11/23 computer, utilizing the ROMP and ROC 3D programs developed by R. Robinson and J. Leary at the University of Rochester.

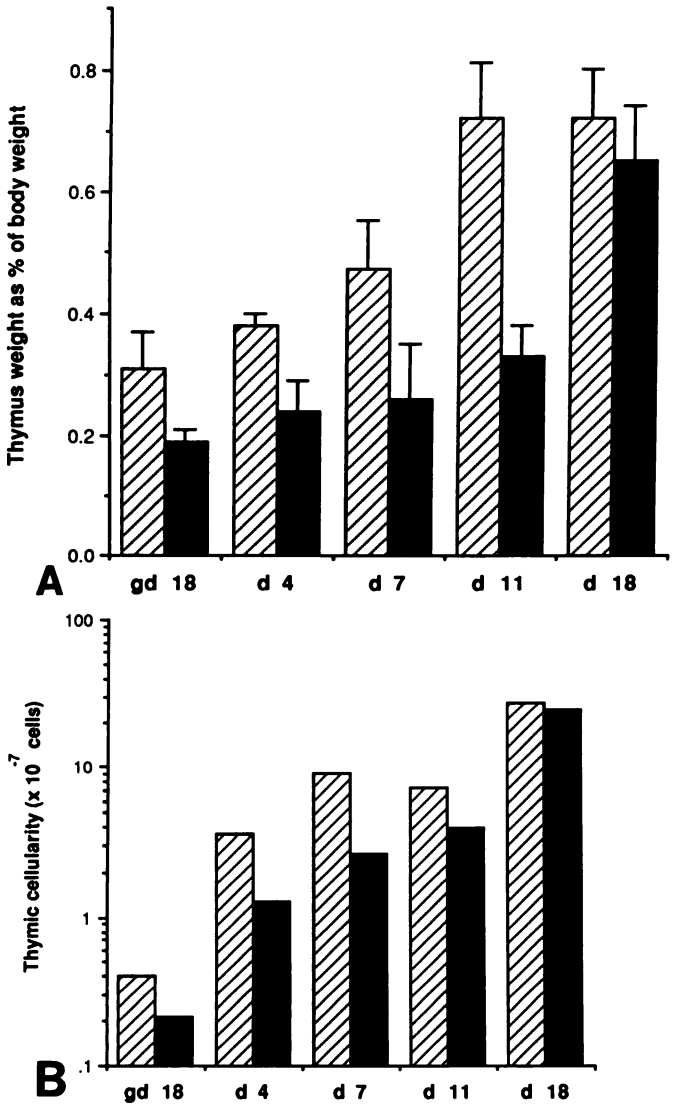

Fig. 1. Thymic atrophy after perinatal TCDD exposure. *gd 18*, gestational day 18; *d4* postnatal day 4; etc. Animals were obtained and pooled as described in Materials and Methods. A, Thymus weight as a percentage of body weight. Values represent the means ± standard deviations of individual mice from 5–15 separate litters from one representative experiment. B, Thymic cellularity from a representative experiment at each time point on a per thymus basis. Values were determined from pools of thymuses from 5–15 litters. At gd 18, control and TCDD cellularity ($\times 10^{-7}$) for two separate experiments were 0.40 and 0.21, respectively, and 0.23 and 0.15; at day 4 (two experiments), 3.55 and 1.28, and 4.10 and 1.85. Experiments on days 7, 11, and 18 were performed once. Note that B is plotted on a semilogarithmic scale. ▨, controls; ■, TCDD-exposed pups.

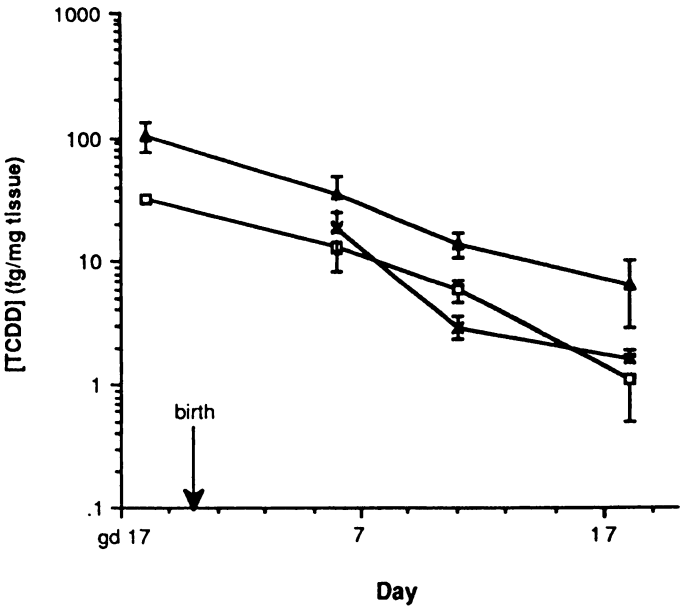

Fig. 2. TCDD concentrations in fetal/neonatal liver (▲), thymus (□), and spleen (×). Values displayed are mean TCDD concentrations ± standard deviations of individuals from two to four litters except for gd 18 thymus, which represents the average concentration from two pooled litters. *Arrow* represents day of birth.

Results

Administration of a single dose of 10 μg of TCDD/kg of body weight to pregnant BALB/c mice, an *Ah*-responsive stain (25), on gd 14 resulted in prominent thymic atrophy, assessed as either thymic weight or as thymic cellularity in both fetuses and neonates (Fig. 1). Thymic atrophy was observed in TCDD-exposed pups as early as gd 18, the earliest time point examined, and was maximal by postnatal days 4–7. By postnatal day 18, atrophy was almost completely reversed. This dose of TCDD did not affect litter size or pup body weight at any time up to postnatal day 18 (data not shown). Administration of [^{3}H] TCDD (2.7 μg/kg) revealed that very low levels of TCDD accumulated in fetal/neonatal thymus, as well as in liver and spleen, with the highest concentrations in each organ observed at gd 18 (Fig. 2). Thereafter, tissue TCDD concentrations decreased with time. Inasmuch as maternal milk is believed to be a major route of exposure for nursing pups (26), it appears that the rapid organ growth in neonatal mice during this period leads to a dilution of tissue TCDD concentrations. This dose of TCDD produced a significant reduction in thymic weight on postnatal day 6 (data not shown). It is worth noting that, in this experiment, thymic TCDD levels ranged from 1 to 31 fg of TCDD/mg of tissue (Fig. 2). These data emphasize the extreme sensitivity of the developing immune system, at least in the mouse, to this compound.

In order to establish whether perinatal TCDD exposure influenced developing lymphocytes, TdT biosynthesis and mRNA levels were examined in bone marrow, thymus, and fetal liver. Values for TdT biosynthesis and mRNA levels have been shown to correlate well with total TdT enzyme activity and the

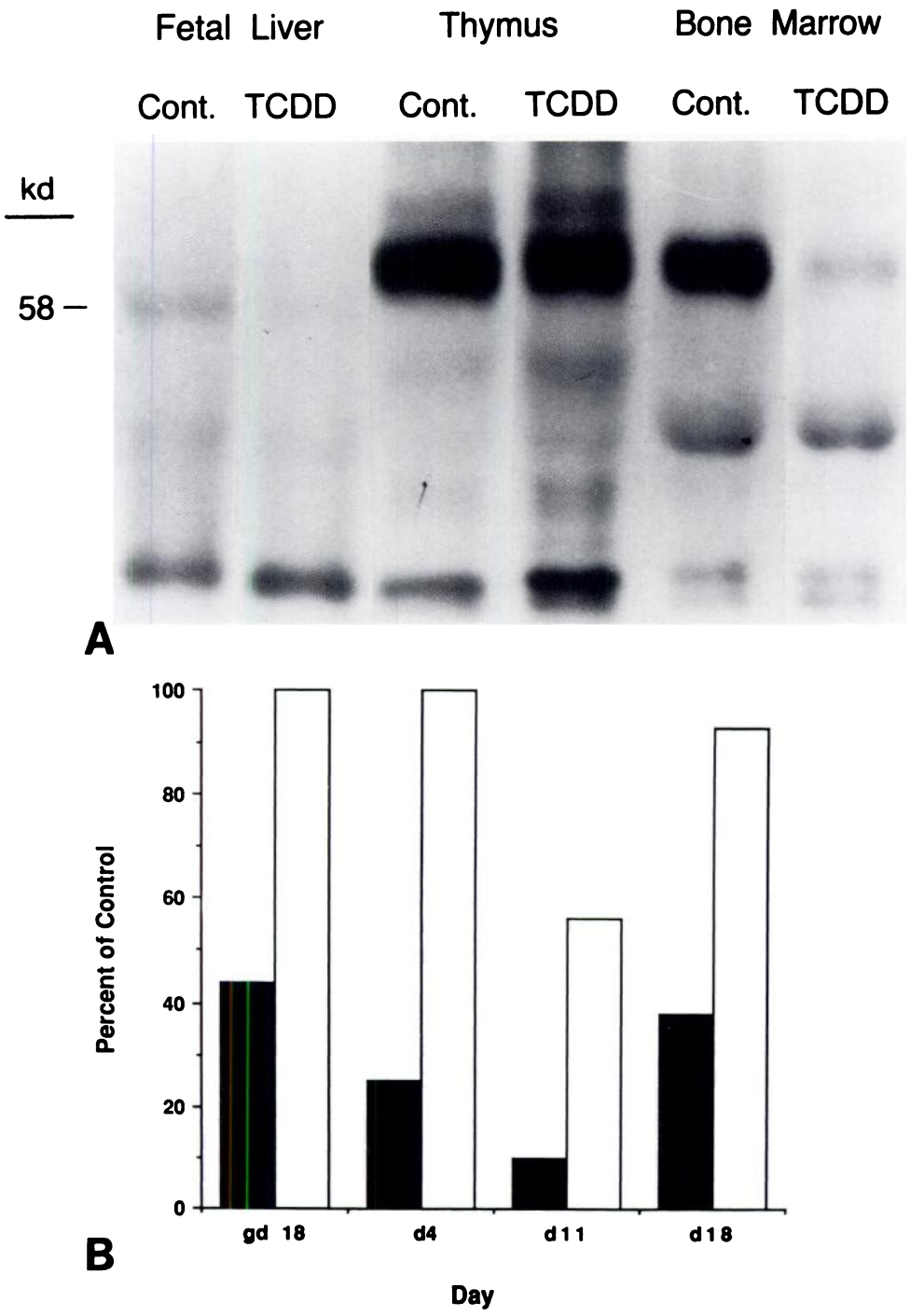

Fig. 3. TdT synthesis in bone marrow, thymus, and fetal liver after perinatal TCDD exposure. A, Anti-TdT immunoprecipitations from thymus and bone marrow from control and TCDD-exposed pups on postnatal day 11 and from fetal liver on gd 18. *Cont.*, vehicle-dosed animals; *TCDD*, TCDD-exposed animals. The 45-kDa actin band can be seen at the bottom of each lane (in bone marrow, actin is the higher molecular weight species of the two bands comprising the doublet at the bottom of each lane). B, TdT synthesis on various days after perinatal TCDD exposure. TdT bands were quantified densitometrically and normalized against the actin band, as described in Materials and Methods. Data are expressed as percent of control. ■, fetal liver/bone marrow; □, thymus.

number of TdT^+ cells observed by immunofluorescence (21, 22, 27) and are quantitatively more specific and sensitive in complex tissues such as bone marrow and fetal liver (21, 22). On gd 18, lymphoid cells from the livers of TCDD-exposed animals had only 40% of the capacity to biosynthesize TdT that control liver lymphocytes from age-matched animals (Fig. 3) did. TdT production in bone marrow cells from treated mice was inhibited from 70 to 90% of control values between postnatal days 4 and 11 (Fig. 3), when TCDD-induced thymic atrophy was most pronounced (Fig. 1), and remained depressed by more than 2-fold on postnatal day 18, when thymic atrophy was virtually reversed. In contrast, the rate of thymocyte TdT synthesis, on a per cell basis, was not significantly suppressed except on postnatal day 11 (Fig. 3B). Reductions in the levels of TdT-specific mRNA as a percentage of total mRNA were also observed and were qualitatively similar to those seen for TdT biosynthesis for these samples (Fig. 4). Thymic TdT-specific mRNA levels were somewhat reduced, although these changes were not nearly as striking as those seen in the marrow and fetal liver.

In light of the above findings on altered lymphocyte stem cell populations, and because some of the toxic effects exerted by TCDD appear to be due to altered cellular proliferation and differentiation (13), it was of interest to determine whether this exposure regimen also affected intrathymic differentiation, as assessed by Lyt-2/L3T4 and IL-2R expression. Murine thy-

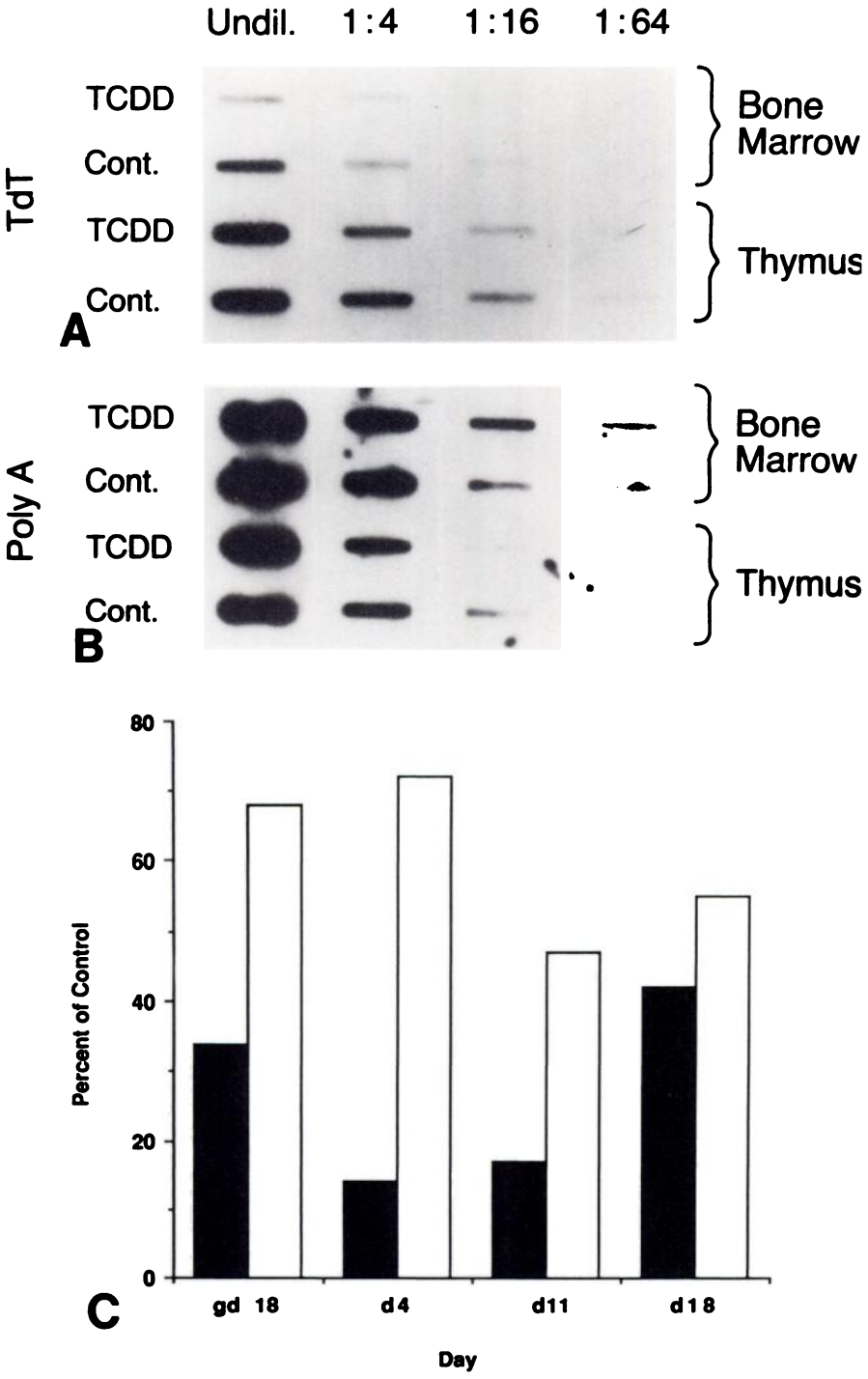

Fig. 4. TdT mRNA levels in bone marrow and thymus after perinatal TCDD exposure. A, TdT-specific mRNA from bone marrow and thymus of vehicle- (*Cont.*) and TCDD-treated (*TCDD*) pups from postnatal day 4. B, Total cellular poly(A) RNA from the same preparation as in A. C, TdT-specific mRNA levels on various days after perinatal TCDD exposure. Densitometric analysis of TdT slots were performed as described in Materials and Methods and these values were normalized to the corresponding poly(A) RNA slot. Data are expressed as percent of control. ■, fetal liver/bone marrow; □, thymus.

mocytes can be separated into four major subsets based on the differential expression of the surface antigens Lyt-2 and L3T4 (4). The Lyt-2$^+$L3T4$^+$ (DP) subset consists of immature cells and appears to include intermediates along the differentiation pathway to the phenotypically mature Lyt-2$^-$L3T4$^+$ and Lyt-2$^+$L3T4$^-$ thymocytes that seed the periphery. The Lyt-2$^-$L3T4$^-$ (DN) subpopulation contains precursors for the other three subpopulations (28). Freshly isolated thymocytes were stained with monoclonal antibodies directed against these surface antigens and analyzed by multiparameter flow cytometry. Table 1 illustrates data from individual experiments that were performed over a period of 6 months. A slight dimunition in the percentage of Lyt-2$^+$L3T4$^+$ thymocytes was observed in two separate experiments in TCDD-exposed fetuses on gd 18, compared with age-matched control fetuses (experiment I and II, *columns 3* and *4*). Small increases in the percentage of Lyt-2$^-$L3T4$^-$ and Lyt-2$^+$L3T4$^-$ thymocytes were observed as well. Similar alterations, along with an increase in the percentage of Lyt-2$^-$L3T4$^+$ cells, were seen on postnatal day 4 (experiments III, IV, and V). By postnatal day 11, the percentage of cells in each subset appeared to be returning to control levels (experiment VI), even though thymic atrophy remained severe (Fig. 1). The percentage of thymocytes expressing IL-2R, thought to demarcate stem-like intrathymic cells that have the capacity

TABLE 1

Thymocyte subpopulations, defined by Lyt-2 and L3T4 expression, after perinatal TCDD exposure

Values are percentage of cells in population. Values represent replicate experiments performed over a total of 6 months. As an additional control to ensure that antibody staining was as expected, normal 5–6-week-old BALB/c mouse thymuses were run with each experiment. Representative percentages of cells in each thymic subpopulation from these mice were Lyt-2$^-$L3T4$^-$, 6.8%; Lyt-2$^+$L3T4$^+$, 76.3%; Lyt-2$^-$L3T4$^+$, 11.6%; Lyt-2$^+$L3T4$^-$, 5.2%.

day[a]	Expt.	Lyt-2$^-$L3T4$^-$		Lyt-2$^+$L3T4$^+$		Lyt-2$^-$L3T4$^+$		Lyt-2$^+$L3T4$^-$	
		Control	TCDD	Control	TCDD	Control	TCDD	Control	TCDD
					%				
gd 18	I	36.7	41.3	53.1	47.0	5.0	4.5	4.2	7.2
	II	24.5	31.5	64.9	52.4	3.8	3.8	6.9	12.4
d4	III	8.3	9.8	70.7	63.5	15.8	20.2	5.3	6.5
	IV	11.4	11.9	64.9	55.4	12.3	19.6	11.4	13.1
	V	10.2	14.3	67.7	58.2	14.5	14.9	7.6	12.6
d11	VI	3.6	4.8	79.8	76.4	11.3	14.0	5.3	4.7

[a] Day of examination.

to further differentiate to mature thymocytes (4), was not altered in TCDD-exposed thymuses at any time examined nor was the mean fluorescence intensity of IL-2R$^+$ cells (data not shown). Despite the absence of major intrathymic alterations with regard to the percentage of cells in these various subpopulations, the number of thymocytes in each of these subsets was reduced in TCDD-exposed mice, due to the extensive thymic atrophy.

Discussion

The present report is the first to demonstrate that the lymphocyte stem cell population in the bone marrow and fetal liver that synthesizes TdT is sensitive to TCDD during the perinatal period. Administration of TCDD to pregnant mice on gd 14 resulted in a greater than 50% reduction in TdT protein synthesis in gd 18 fetal liver and a 70–90% reduction in neonatal bone marrow (Fig. 3). Similar reductions in TdT mRNA levels were observed as well (Fig. 4). This lesion persisted through postnatal day 18, when thymic atrophy was recovered. In contrast, the thymic TdT-synthesizing stem cell population appears to be generated normally on a per cell basis, although the actual number of these cells was diminished due to thymic atrophy. This phenomenon is distinct from the reduction in the TdT$^+$ cell populations observed after corticosteroid (7, 9) or radiation (29) treatment, in which there is selective and coordinate ablation of both marrow and thymus TdT-synthesizing cells. This suggests that TCDD may be acting via a different mechanism, at least during this perinatal period, than either of these agents. The possibility that the effects of TCDD on TdT-synthesizing stem cells may be dose dependent cannot yet be ruled out, however. Nevertheless, it appears that the TdT-synthesizing fetal liver/bone marrow lymphocyte stem cell is especially sensitive to TCDD during immune system ontogenesis (at the doses used here), compared with the thymic cell that synthesizes TdT.

After a single dose of TCDD on gd 14, we observed thymic atrophy by gd 18. This atrophy was maximal by postnatal day 4–7 and was virtually recovered by postnatal day 18 (Fig. 1). Thymic atrophy was observed in mice after weaning following a perinatal multiple dosing regimen (19) and was seen for as long as 145 days after birth in rats exposed to TCDD via maternal dosing pre- and postnatally, whereas rats exposed only postnatally displayed atrophy only until 39 days after birth (30). The reasons for the differences between these and the present studies may be related to the different dosing schedules and/or the different species or mouse strains used.

TCDD has been reported to alter cell differentiation and proliferation in a number of tissue and cell types (reviewed in Ref. 13), and administration of TCDD to primary cultures of murine and human thymic epithelial cell monolayers has been reported to suppress the proliferative response of syngeneic thymocytes to concanavalin A and phytohemagglutinin, implying an impairment of thymic differentiation processes by TCDD (15, 31). However, we found that thymocyte differentiation, determined by staining cells with antibodies directed against Lyt-2, L3T4, and IL-2R, was not profoundly altered after perinatal TCDD exposure. We observed by flow cytometric analysis a slight reduction in the percentage of cells in the DP subpopulation on gd 18 and on postnatal day 4, with slight increases in the Lyt-2$^-$L3T4$^-$, Lyt-2$^-$L3T4$^+$, and Lyt-2$^+$L3T4$^-$ subsets (Table 1). By postnatal day 11 these differences were reduced, despite the fact that the thymus remained atrophied and that TdT synthesis and mRNA levels were greatly reduced in bone marrow cells. The number of cells in each subset in exposed animals was markedly diminished throughout this time period, however, due to the reduced size of the thymus (until postnatal day 18 when the thymus returned to normal size). The lack of notable alterations in thymic subsets described here highlights another difference between corticosteroid treatment and exposure to TCDD, in that the former produces a rapid and severe reduction in DP thymocytes and a dramatic increase in the mature, single positive subsets (32). This may be due to the differential degree of thymic atrophy seen after corticosteroid exposure (33) compared with TCDD and/or the different mechanism of action of these compounds. That dexamethasone and TCDD appear to inhibit *in vitro* B lymphocyte maturation differently (34) lends support to the latter possibility.

No differences were observed in the percentage of IL-2R$^+$ thymocytes at any time, althugh the absolute number of IL-2R-expressing cells was reduced because of atrophy. IL-2R is expressed on a subset of DN thymocytes (4), and thymocytes that express IL-2R appear to be important intermediates in the differentiation into phenotypically mature T lymphocytes (35). IL-2R-bearing thymocytes have also been shown to play an important role in the generation of cells bearing $\alpha\beta$ T cell receptor heterodimers in fetal thymic organ cultures (36). These data have suggested an integral role for IL-2R$^+$ thymocytes in intrathymic development. However, the absence of severe and prolonged alterations in these thymic subpopulations (with regard to Lyt-2/L3T4 and IL-2R expression), in concordance with the aforementioned TdT results, suggests that TCDD-induced thymic atrophy, at least during the perinatal period, is not linked to any major alterations in intrathymic developmental patterns. It remains possible that the rapid cellular differentiation and proliferation that occur in the thymus during this period may obscure subtle changes in the pattern of marker expression, expecially in small subsets of intrathymic stem cells. An alternative hypothesis, which we do not favor inasmuch as the various subpopulations may be differentially regulated, is that perinatal TCDD exposure may retard thymocyte development at all points along the differentiation pathway, resulting in no observable changes in the percentage of cells in each subset.

The findings reported here indicate that the lymphocyte stem cell is a target of TCDD, at least during perinatal development, and suggest the utility of this compound as a probe to further examine the role of the *Ah* receptor in the development of the immune system, as well as to gain a better understanding of the molecular basis by which TCDD induces immunosuppression. Because both pre-B cells and prothymocytes synthesize TdT (5–10), the fact remains that perinatal TCDD administration may result in a reduction in one or both subsets. Although we are not ruling out an effect on TdT-synthesizing pre-B cells, the degree of suppression of TdT biosynthesis and its temporal correlation with thymic atrophy is consistent with a reduction in the number of fetal liver/marrow prothymocytes. Thus, the observed TCDD-induced thymic atrophy may be explained by a reduced rate of thymic seeding by precursor cells from both fetal liver and bone marrow. Notably, we have observed that TCDD administration to adult mice also causes a reduction in TdT biosynthesis that is qualitatively similar to that described above after perinatal TCDD exposure. Bone marrow cells from these adult mice demonstrate impaired capacity for thymic regeneration and reduced CFU-S numbers.[1] In light of the reported differences in the sensitivity of the perinatal and adult thymus and cell-mediated immune system to TCDD (reviewed in Ref. 18), it will be of interest to determine whether prothymocytes from perinatally exposed animals are similarly influenced. These investigations are currently in progress.

In summary, the observations presented here are consistent with the hypothesis that perinatal TCDD exposure produces thymic atrophy by reducing the number of prethymic stem cells, resulting in decreased thymic seeding. Thymic TdT production (on a per cell basis) appears to be generally unaffected by TCDD exposure. Intrathymic development, assessed by surface Lyt-2/L3T4 and IL-2R expression, is slightly modified soon after TCDD administration but appears to be normal shortly after birth, when thymic size and marrow TdT levels remain depressed. These data suggest that those prothymocytes that do arrive at the thymus develop relatively normally, although the total number of thymocytes in each subset is reduced due to extensive thymic atrophy. Thus, thymic processing appears to be occurring, for the most part, properly in these animals, although on a reduced number of cells, suggesting that the reported alterations in cell-mediated immune function (18, 19) may be due, in part, to reduced thymic output of mature immunocompetent T lymphocytes.

Acknowledgments

We thank Dr. Allen Gates and Katie Maczynski of the Environmental Health Sciences Center's Inbred Mouse Unit for breeding mice and Drs. Martin Yuille, Jacob Finkelstein, and Ellen Henry for helpful discussions. We are grateful for the technical expertise of Margaret Hugunin and Dr. Xinming Zhang. We also acknowledge Dr. James Leary and Scott McLaughlin for their advice and technical assistance with the flow cytometric analyses.

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