INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is considered the most toxic congener of a class of related compounds, the halogenated aromatic hydrocarbons. One of the toxic manifestations of exposure in many species is a wasting syndrome. These animals exhibit prolonged weight loss due to depletion of both lean and adipose tissue mass, the basic mechanism of which remains to be established. Among the biochemical changes in adipose tissue that accompany wasting are decreased lipoprotein lipase activity with associated serum hyperlipidemia (Brewster and Matsumura, 1984), decreased glucose uptake, most likely due to effects on GLUT-4 activity (Enan et al., 1992; Olsen et al., 1994), and changes in protein phosphorylation (Enan and Matsumura, 1993, 1994). Collectively, these observations point to the adipocyte as a major target for TCDD-induced toxicity.

Most, if not all, of the diverse effects of TCDD and related compounds are believed to be mediated by their interaction with a soluble receptor, the Ah receptor or AhR, which upon ligand binding becomes localized to the nucleus (Poland and Knutson, 1982; Whitlock, 1990). There it associates with the nuclear protein, arnt (Reyes et al., 1992; Whitelaw et al., 1993), and the complex binds to dioxin-response elements (Denison et al., 1988) to activate transcription. This receptor may also play a role in some of the non-transcriptional effects of TCDD (Enan and Matsumura, 1994). Cloning and sequencing of the AhR and arnt have shown them to be members of the basic/helix-loop-helix family of transcription factors (Burbach et al., 1992; Hoffman et al., 1991).

The preadipose 3T3 clonal cell lines, 3T3-L1 (Green and Kehinde, 1974) and 3T3-F442A (Green and Kehinde, 1976), are convenient cell culture models both for investigation of the program of adipose differentiation and for determination of factors that regulate the physiology of the mature adipocyte. Quiescent cells can be induced to differentiate by the addition of various hormones or drugs in the presence of adipogenic factors that are present in fetal bovine serum. During this process there is extensive induction of mRNA and protein to enable the cell to synthesize and store large amounts of triglyceride (for reviews see Green, 1978; Ailhaud et al., 1992; Spiegelman et al., 1993).

A number of inhibitors of adipose differentiation have been identified, such as TGFβ (Ignotz and Massague, 1985), FGF and PDGF (Hayashi et al., 1981), phorbol ester (Diamond et al., 1977), retinoic acid (Kuri-Harcuch, 1982), TNFα (Torti et al., 1985, 1989) and other cytokines (Gregoire et al., 1992). The relationships among these inhibitors, their mechanisms of action and their roles in adipocyte homeostasis are largely unknown. Since animals exposed to TCDD exhibit a dramatic reduction of adipose tissue mass, it seemed possible that TCDD would be a potent inhibitor of the differentiation process, a hypothesis that has been tested here.

SUMMARY

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced toxicity is particularly striking in adipose tissue, where it causes severe wasting. This phenomenon suggests that TCDD could have effects on adipocyte differentiation, now demonstrated using 3T3-L1 cells as a model system. When cells were treated with 10 nM TCDD before differentiation or during the first two days of induction in the presence of dexamethasone (dex) and isobutylmethylxanthine (IBMX), a reduction occurred in the number of fat cell colonies measured 7-10 days later by Oil Red O staining. Northern blotting showed an accompanying reduction in amounts of mRNA encoding several adipocyte markers. In contrast, when TCDD was added after differentiation, it had no effect on the maintenance of the adipose phenotype. Dose-response and structure-activity relationships were consistent with a process mediated by interaction of TCDD with the Ah receptor. The possibility that TCDD acts by inhibiting the signaling pathways activated by dex and IBMX was investigated. TCDD did not interfere with glucocorticoid-inducible transcription as judged by the unimpaired responsiveness of a transfected reporter construct. Treatment of cells with TCDD augmented the increase in protein kinase A (PKA) activity elicited by either IBMX or forskolin; therefore, if TCDD disrupts the cAMP signaling pathway, it must do so at a step after activation of PKA.

Key words: TCDD, adipocyte, differentiation
MATERIALS AND METHODS

Cell cultures

3T3-L1 cells (Green and Kehinde, 1974), obtained from ATCC, were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% bovine serum and passed just before confluence. For differentiation experiments, cultures were maintained for 1-2 days at confluence, then switched to differentiation medium (a mixture of DMEM and Ham’s F12 medium (3:1) supplemented with 10% fetal bovine serum, 10 μg/ml insulin, 5 μg/ml transferrin, 20 μM triiodothyronine and 0.18 mM adenosine). For the first 3 days of differentiation induction, the medium contained two additional inducers, dexamethasone (dex; 1 μM) and isobutylmethylxanthine (IBMX; 0.2 mM) (Russell and Ho, 1976; Rubin et al., 1978). After this time cells were cultivated in differentiation medium without inducers and fresh medium was added every 2 days. About 2 days after removal of dex and IBMX, adipocyte colonies begin to be visible as regions containing rounded cells with numerous intracellular lipid droplets. Chemicals to be tested were prepared as 1000x stocks in dimethylsulfoxide or ethanol. Addition of any of these solvents alone, examined in parallel, had no apparent effect on differentiation.

Oil Red O staining

When differentiation was complete, as judged by visual inspection, cultures were fixed with 10% formalin in PBS for 1 hour or more, then rinsed 3 times with distilled water and the dishes were air dried. Staining with Oil Red O was performed as described (Kuri-Harcuch et al., 1978). In some experiments staining was quantitated spectrophotometrically (Kasturi and Joshi, 1982).

Glycerophosphate dehydrogenase (GPD) assays

Cells grown in 60 mm culture dishes were rinsed 2 times with phosphate-buffered saline (PBS), scraped into 0.2 ml of 25 mM Tris-HCl, pH 7.5, 1 mM EDTA and sonicated. The resulting extract was clarified by centrifugation in a microfuge for 10 minutes at 4°C and was frozen at −80°C. GPD activity was determined spectrophotometrically by measuring the oxidation of NADH in the presence of dihydroxyacetone phosphate (Wise and Green, 1979). Protein was determined by the Bradford (1976) method using the Bio-Rad protein reagent.

RNA extraction and analysis

Cell cultures grown in 10 cm dishes were rinsed twice with PBS and solubilized with 5 M guanidine thiocyanate containing 1% β-mercaptoethanol. RNA was isolated by centrifugation through 5.7 M CsCl (Chirgwin et al., 1979). Purified total RNA, dissolved in water and quantitated by absorbance at 260 nm, was analyzed by agarose gel electrophoresis and northern blotting (Fourney et al., 1988). Blots were probed overnight with 32P-labeled cDNA probes in a solution containing 1 M NaCl, 100 mM sodium phosphate, pH 6.5, 10 mM EDTA, 1% SDS, 100 μg/ml yeast tRNA, and 200 μg/ml sheared, denatured salmon sperm DNA, and then were hybridized with 32P-labeled cDNA probes in the same solution. Blots were washed with 2× SSC, 0.1% SDS at room temperature followed by 1× washes at 65°C with 0.5% SSC, 0.1% SDS. The cDNA probes for GPD, adipocyte fatty acid binding protein (FABP), adipin and β-actin (Spiegelman et al., 1983) were obtained from Dr Howard Green (Harvard Medical School). The probe for murine lipoprotein lipase (LPL) was generated by RT-PCR of a 900 bp region using primers based on published sequence (Kirchgessner et al., 1987). Probes were labeled by the random primers method (Feinberg and Vogelstein, 1983) with [α-32P]dCTP (Amersham) using a Pharmacia kit.

Protein kinase A assays

Protein kinase A (PKA) activity was determined in cell lysates using a kit from Gibco/BRL. Briefly, 10 cm cultures were rinsed with PBS, scraped into 1 ml of 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and homogenized (10 strokes in a Dounce homogenizer). Insoluble material was removed by centrifugation and the supernatant was assayed in the presence of [γ-32P]ATP using synthetic Kemptide at 50 μM as substrate. Total PKA activity was measured by addition of 10 μM cAMP and basal activity, a measure of active PKA at the time of harvest, was measured in the absence of exogenous cAMP. Unincorporated 32P was removed by spotting samples on phosphocellulose paper and washing with dilute H3PO4. Nonspecific activity, determined in the presence of 1 μM PKI(6-22)amide, was subtracted.

Transfection assays

The glucocorticoid-responsive MMTV-LTR (plus and minus the RSV enhancer) was inserted upstream of the luciferase coding region of pGL2-basic (Promega) to make MMTV-LUC and RSV/MMTV-LUC. The plasmids were transformed into bacteria using standard techniques and plasmid DNA was purified using a Promega Magic Prep kit. Confluent 3T3-L1 cultures in 60 mm dishes were transfected with 10 μg of plasmid DNA by a modification (Graves et al., 1991) of the calcium phosphate method. Briefly, on the day of transfection, cultures were rinsed with 1 mM EDTA in PBS, then refed with growth medium. Three hours later, the DNA/calcium phosphate coprecipitate was added. After another 3 hour incubation, culture medium was removed and cells were exposed for 3 minutes to 10% glycerol in PBS. At this time culture medium with differentiation inducers plus TCDD or solvent was added and cells were returned to the incubator for 2 days. Luciferase was assayed using a kit from Promega.

RESULTS

TCDD suppresses morphological differentiation of 3T3-L1 cells

When confluent (day 0) 3T3-L1 (Green and Kehinde, 1974; Green and Meuth, 1974) are treated for 3 days with inducing agents, dex and IBMX (Russell and Ho, 1976; Rubin et al., 1978), in the presence of fetal bovine serum and insulin, then subsequently cultivated in the absence of inducers, they accumulate prominent lipid droplets that can be stained with Oil Red O. Treatment of cells with TCDD during this time suppressed the number of colonies that stained for lipid (Fig. 1). The effect was most pronounced if TCDD was added before or during the first 2 days of dex and IBMX treatment. Later addition was ineffective; consequently, TCDD showed no

Fig. 1. Effect of time of TCDD treatment on adipocyte differentiation. Cells were treated with 10 nM TCDD 3 days before addition of dex/IBMX (−3), at the time of dex/IBMX addition (0) or one (1) or two (2) days after dex/IBMX addition. In this experiment TCDD was added at every medium change. Cultures were fixed and stained with Oil Red O.
ability to reverse the adipocyte phenotype once it had been established, even with prolonged treatment (not shown).

Short treatments with TCDD (as short as 1 hour), followed by a medium change, were as effective in suppressing the adipocyte phenotype as when the toxin was present continuously; however, the lipophilicity and low rate of metabolism of TCDD make it difficult to remove it effectively. Since this result may well reflect its continued presence, a minimal time required for its action cannot be assessed. For practical purposes, TCDD was added to cultures during the treatment with dex and IBMX, and removed when cells were switched to medium with fetal bovine serum and insulin alone.

The extent of suppression of differentiation by TCDD was estimated by assaying GPD activity, which has been shown in most cases to correlate well with differentiation, and by spectrophotometric determination of Oil Red O staining after elution from stained dishes. In both cases, values from TCDD-treated dishes were approximately 20% of control (untreated) values (GPD, 20±19% in 7 experiments; Oil Red O, 20±3% in 3 experiments). The results from GPD assays were more variable and in some experiments appeared higher than expected, based on Oil Red O staining of replicate dishes. One possibility is that TCDD has multiple effects: in addition to its ability to suppress differentiation it may affect GPD transcription in those cells that escape suppression and are able to differentiate.

Suppression by TCDD did not occur in all preadipose 3T3 lines; in 3T3-F442A (Green and Kehinde, 1976), TCDD slowed the differentiation process, but was unable to prevent it from ultimately occurring. An intriguing possible explanation is that TCDD titrates (inactivates) some critical differentiation factor present at varying levels, perhaps the factor that was selected in the derivation of these preadipocyte clones (Green and Kehinde, 1976). TCDD also had no obvious effect on growing, preadipose 3T3 cells. Treated and untreated cultures were indistinguishable morphologically and grew at identical rates, both having a doubling time of approximately 15 hours.

**TCDD suppresses accumulation of adipocyte-specific mRNAs**

Adipose differentiation of 3T3 cells can occur without lipid accumulation under certain conditions (e.g. biotin deficiency; Kuri-Harcuch et al., 1978). To ensure that TCDD action was due to inhibition of differentiation, the effect of TCDD treatment on accumulation of adipocyte-specific mRNAs was determined. Cells were differentiated in the presence or absence of 10 nM TCDD and RNA was prepared. Northern blots were probed with cDNAs to glycerophosphate dehydrogenase (GPD), adipocyte fatty acid binding protein (FABP) (also referred to as aP2 [Cook et al., 1985] or 422 [Bernlohr et al., 1984]), lipoprotein lipase (LPL) and adipsin, all of which greatly increase in adipocytes, and with cDNA to actin, which decreases (Spiegelman et al., 1983). All of the adipocyte-specific mRNAs were reduced in TCDD-treated cultures and the usual differentiation-dependent decrease in actin mRNA levels was observed in untreated but not TCDD-treated cultures (Fig. 2, lanes 1 and 2). Detectable levels of adipocyte mRNAs were expected in treated samples, since TCDD does not totally eliminate fat cell colonies, but greatly reduces their number. Of particular interest is the suppression of LPL mRNA, which has been reported to increase at confluence without the necessity for the presence of adipogenic factors (Dani et al., 1990). This suggests that TCDD is influencing a very early step in the differentiation pathway. In agreement with the results from staining for triglyceride, mRNA levels were unchanged by TCDD compared to the solvent control when these agents were added after differentiation was complete (Fig. 2, lanes 3 and 4).

**Structure activity relationship**

Many, if not all, of the actions of TCDD depend upon its binding to a soluble receptor. Other polyaromatic hydrocarbons are also ligands for this receptor and a rank order of potency has been determined for both binding and biological activity. Although TCDD binding activity in these cells has not yet been reported, they have been shown to respond in terms of decreases in glucose uptake (Olsen et al., 1994) when exposed to nanomolar concentrations of TCDD. Consistent with receptor mediation of the suppression of adipocyte differentiation by TCDD, the effect is dose dependent with an EC50 between 10^{-11} and 10^{-10} M (Fig. 3). In addition, three other polyaromatic hydrocarbons were tested, two of which bind with less avidity to the receptor, 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PCDD) and 3,3′,4,4′-tetrachlorobiphenyl
Fig. 3. Dose of TCDD required to suppress differentiation. Cells were treated with the indicated molar concentrations of TCDD during the induction period with dex/IBMX, then incubated in the absence of the toxin until fat cell colonies were prominent in the culture treated with solvent alone (0). Cultures were then fixed and stained with Oil Red O.

Fig. 4. Effect of various halogenated aromatic hydrocarbons on adipocyte differentiation. Cultures were treated with the indicated doses of the indicated compounds and stained as described for Fig. 3.

(3′,4′,4′-TCB) and one which is not a ligand, 2′,5′,5′-tetrachlorobiphenyl (2′,2′,5′-TCB) (Fig. 4). Each compound was tested at two concentrations in parallel with TCDD. Cells were fixed on day 7 and stained with Oil Red O. 1,2,3,7,8-PeCDD, which has about a 10-fold higher Kd than TCDD, required 100 nM to produce approximately the same effect as that produced by 10 nM TCDD. 3′,4′-TCB suppressed differentiation, but to a lesser extent than TCDD even when used at a 10-fold higher concentration. As predicted, 2′,2′,5′-TCB had no effect on differentiation.

α-Naphthoflavone (αNF) has been shown by others to act as a partial TCDD antagonist at concentrations of 1 µM or less, although it exhibits agonist activity at higher concentrations (Gasiewicz and Rucci, 1991; Santostefano et al., 1993). This has been shown to occur because αNF competes with TCDD for binding to the AhR, but is unable to efficiently transform the receptor to its DNA-binding form. Table 1 shows that at a 1000-fold molar excess, αNF could partially block the TCDD-induced inhibition of fat cell differentiation. It is possible that the lack of complete reversal of TCDD action is due to the long time period required for the differentiation process to be manifest and to the susceptibility of αNF, but not TCDD, to metabolism.

**Effect of TCDD on metabolism of inducers**

Since TCDD is a very potent inducer of enzymes involved in metabolizing polyaromatic hydrocarbons, it may produce its effect on fat cell differentiation by metabolizing IBMX or dex or both. Two substitutes for IBMX were tested, 8Br-cAMP and forskolin, which raise intracellular cAMP levels by different means and have little structural similarity to IBMX. Both worked as well or better than IBMX in initiating differentiation and in both cases differentiation was suppressed by addition of TCDD (Fig. 5).

To assess the possible effects of TCDD treatment on glucocorticoid metabolism, the EC50 values were determined for dexamethasone induction of GPD activity (a measure of the number of differentiated cells; Pairault and Green, 1979) in the presence and absence of TCDD. This is possible because a low level of differentiation occurs in the presence of TCDD. If TCDD were to block differentiation by inducing metabolism of dex, there should be a substantial shift in the dose-response curve and a resultant increase in the EC50. Fig. 6 shows that although the levels of GPD activity were lower at every dose of glucocorticoid in the presence of TCDD, activity was still glucocorticoid-dependent and the EC50 values were close for treated and untreated cultures (7×10⁻⁸ M and 5×10⁻⁸ M, respectively).

**Table 1. Effect of AhR antagonist on adipose differentiation as assessed by GPD activity**

<table>
<thead>
<tr>
<th>Treatment/TCDD</th>
<th>GPD activity (U/mg)</th>
<th>Treatment/DMSO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>337</td>
<td></td>
</tr>
<tr>
<td>TCDD, 10⁻⁹ M</td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>αNF, 10⁻⁶ M</td>
<td>314</td>
<td>93</td>
</tr>
<tr>
<td>αNF, 10⁻⁶ M + TCDD, 10⁻⁹ M</td>
<td>204</td>
<td>61</td>
</tr>
</tbody>
</table>

*Cells were treated at the time of the switch to differentiation medium with dex and IBMX. When cells were treated with both TCDD and αNF, the latter was added 1 hour ahead. After 3 days, medium was changed to FBS plus insulin (as described in Materials and Methods). TCDD was removed at this time, since continuous treatment was shown to be unnecessary. In contrast, continuous treatment with αNF was required in order to be effective in blocking TCDD effects. The difference is probably due to the very low rate of metabolism of TCDD as compared with other polychlorinated aromatic hydrocarbons. Cells were harvested on day 7 for GPD assays.

†1U is the amount of enzyme that will oxidize 1 nmol NADH/min.

**Effect of TCDD on glucocorticoid and cAMP signaling pathways**

Although TCDD does not suppress differentiation by metabolically inactivating the IBMX or dex, it could interfere with a crucial step in the signaling pathways. The integrity of the glucocorticoid response pathway was tested by transfecting cells with two glucocorticoid-responsive reporter constructs,
TCDD inhibits adipocyte differentiation

Fig. 5. TCDD suppression of adipocyte differentiation induced by various agents that increase cAMP. Cultures were treated with 10 nM TCDD or DMSO (solvent control) as described for Fig. 3, except that different agents were used to elevate cAMP at the following concentrations: 2 µg/ml forskolin; 1 mM 8-Br-cAMP; or 0.2 mM IBMX.

Fig. 6. Effect of TCDD treatment on the dose-response relationship for glucocorticoid induction of differentiation. Cultures were treated with 10 nM TCDD or with solvent (no TCDD) as for Fig. 3 but with the indicated doses of dexamethasone. When prominent fat cell colonies were visible, cultures were harvested for GPD assay to quantify the extent of differentiation. 1 U, the amount of enzyme that oxidized 1 nmol NADH/min.

MMTV-LUC and RSV/MMTV-LUC, which were assayed in the presence and absence of TCDD. Similar results were obtained with both plasmids (Fig. 7). Dexamethasone increased luciferase activity approximately 15-fold, which was unaffected by TCDD.

Although IBMX has multiple effects, it can be substituted with other more selective agents that act primarily to raise cAMP concentrations (Fig. 5) and (Williams and Polakis, 1977; Elks and Manganiello, 1985; Cook et al., 1988), suggesting an important role for cAMP and activated protein kinase A (PKA) in the differentiation process. This, coupled with the demonstrated early effects of TCDD on the activities of a number of protein kinases (Matsumura, 1994), led us to examine PKA activities in differentiating cells with and without TCDD treatment. In experiment 1 of Table 2, PKA activity was measured after addition of IBMX, since this is the agent routinely used as inducer for differentiation. The activity ratio (proportion of total PKA that is active in the cell) after 1 hour of treatment with IBMX was low (0.9%) and was increased 2-fold in the presence of TCDD (2%); after 24 hours no activated PKA was detectable above background levels although total activity remained largely unaffected. The effect of TCDD on forskolin-induced PKA activity at 1 and 24 hours was also determined, since forskolin can be substituted for IBMX as an adipogenic inducer and is more effective for raising intracellular cAMP concentrations, thereby eliciting higher PKA activity ratios. Total PKA was lower in this experiment but was largely unaffected by any treatment, and, as expected, activity ratios were much higher. Again, TCDD increased the activity ratio about 2-fold when measured 1 hour after addition of agents. At 24 hours, activity was greatly diminished, although detectable, in both treatment groups, which were not very different from each other. The increased PKA activity at 1 hour in TCDD-treated cultures compared to
untreated cultures was reproducible but seems unlikely to explain the ultimate differences in differentiation, since IBMX and forskolin produced similar numbers of fat cell colonies (Fig. 5), while PKA activity ratios differed about 10-fold. Moreover, forskolin-treated cultures that were not exposed to TCDD had a higher PKA activity ratio than cultures treated with both IBMX and TCDD; the former differentiated well while the latter did not. It is possible that the cAMP signaling pathway might be affected downstream of PKA, but the observation that TCDD can also diminish the lesser amount of differentiation that is produced in the absence of IBMX or IBMX plus dexamethasone (not shown) is consistent with an effect that is independent of these agents.

### DISCUSSION

We have shown that addition of TCDD to cultures of 3T3-L1 cells within a window of time during the induction of differentiation by dexamethasone and IBMX diminished the number of fat cell colonies formed. This effect was dose dependent and appeared to be mediated by the Ah receptor. One possibility is that TCDD interfered with the action of dexamethasone and IBMX, especially since it was ineffective in suppressing differentiation of 3T3-F442A cells, which do not respond to these two inducers. Although TCDD-treated cells were still able to respond to glucocorticoids by transcriptional induction of a GRE-dependent reporter gene, it is possible that TCDD directly suppressed the activity of a glucocorticoid-induced differentiation factor, such as CEB/Pβ (Cao et al., 1991).

To determine whether TCDD acts by disruption of the cAMP signaling pathway, we treated and untreated extracts was eliminated by 24 hours in spite of the continued presence of TCDD. The ability of TCDD to affect PKA activity was not unexpected, since several groups have reported that treatment of animals resulted in increased adipose tissue PKA activity (Enan and Matsumura, 1993) or hepatic cAMP levels (Stahl et al., 1993). The latter group observed that in contrast to the usual effect of increased cAMP to increase PEPT2 mRNA, TCDD treatment resulted in lower levels of this mRNA, which was attributed to decreased ability to respond to the cAMP signal. A decrease in responsiveness to cAMP was also observed in heart from TCDD-treated animals (Canga et al., 1988).

TCDD could also disrupt signaling pathways activated by other adipogenic factors present in the culture medium, such as insulin or IGF-I, or unidentified components of fetal bovine serum. Of special interest are IGF-I, which has been demonstrated to play an important role in the generation of adipocytes (Zezulak and Green, 1986; Smith et al., 1988), and EGF, which under serum-free culture conditions augments the adipogenic activity of IGF-I (Schmidt et al., 1990). Since EGF receptor binding activity is drastically reduced in many cell types by TCDD treatment (Kaelin et al., 1983; Madhusuk et al., 1984; Hudson et al., 1985), this potential target warrants further investigation.

The relationship of the anti-adipogenic activity of TCDD to that of other agents is an open question. Its action may be different in some respects from that of TNF and TGFβ, which induced the de-differentiation of TA1 adipocytes (Torti et al., 1989). Others, however, using the 3T3 preadipocyte lines, have reported that TGFβ and TNF, like TCDD, must act early in the differentiation program to have an effect, and were unable to reverse the process if added later (Ignatov and Massague, 1985; Hotamisligil et al., 1993). Since adipose tissue contains TNF mRNA and secretes immunologically detectable TNF (Hotamisligil et al., 1993), it is possible that TCDD acts in an autocrine fashion to increase production of TNF and/or some other cytokine. In this regard, TNF alone or in combination with other cytokines (Spiegelman and Hotamisligil, 1993) and TCDD produce a similar pathophysiological state in animals, which includes muscle and adipose tissue wasting, serum hyperlipidemia and decreased LPL activity. In addition, there is evidence that some aspects of TCDD-induced toxicity in animals may be mediated by TNF (Taylor et al., 1992).

While the precise mechanism by which TCDD prevents differentiation of 3T3-L1 cells remains elusive, we have established that TCDD acts only during the very early period of differentiation and is unable to reverse the process once it is established. We have further shown that this action of TCDD is mediated by the Ah receptor.

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