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Aryl Hydrocarbon Receptor-Dependent Suppression by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin of IgM Secretion in Activated B Cells

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ABSTRACT

The immune system has been identified as a sensitive target for the toxic effects produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Furthermore, the B cell has been identified as a sensitive cellular target of TCDD by previous cell-type fractionation studies from this laboratory. The mechanism responsible for the immunotoxic effects produced by TCDD is unclear; however, many of the biological effects of TCDD are thought to be mediated by the aryl hydrocarbon receptor (AhR). Here, we describe two B cell lines that differ considerably in their expression of the AhR and in their sensitivity to TCDD. Our results demonstrated a marked expression of the AhR protein in the CH12.LX B cell line but not in the BCL-1 B cell line. Transcripts for the AhR were not detected by reverse transcriptase-polymerase chain reaction in the BCL-1 cells. The AhR nuclear translocator (ARNT) protein was highly expressed in both cell lines. In addition, the AhR and ARNT are functional in CH12.LX cells as demonstrated by TCDD-induced CYP1A1 induction. TCDD did not induce CYP1A1 in BCL-1 cells. Furthermore, TCDD treatment resulted in suppression of lipopolysaccharide (LPS)-induced IgM secretion in CH12.LX cells. Conversely, TCDD-induced inhibition of IgM secretion was not demonstrated in LPS-stimulated BCL-1 cells, implicating a role for the AhR in the inhibition of B cell effector function. LPS-induced differentiation of the CH12.LX cells also resulted in a marked induction of Ahr expression which was not induced in LPS-stimulated BCL-1 cells. These studies have implicated the AhR as a critical factor in TCDD-induced inhibition of IgM secretion and have demonstrated an induction of AhR gene and protein expression after B cell activation.

HAHs such as the polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls are persistent environmental toxins. TCDD has been considered the prototype of HAHs because of its biological potency in experimental animals. A plethora of biological effects have been observed in most animal models after exposure to TCDD. Of these effects, which include a general wasting syndrome, lymphoid involution (especially of the thymus), hepatotoxicity, tumor promotion, and reproductive and developmental toxicity (reviewed in Poland and Knutson, 1982), immune suppression seems to be one of the most sensitive consequences of TCDD exposure. Effects of TCDD on immunocompetence have been well documented in virtually every species studied and occur at doses that do not produce obvious signs of toxicity (reviewed in Holsapple *et al.*, 1991). Although TCDD-induced alterations of innate, cell-mediated, and humoral immunity have been observed, a major cellular component of humoral immunity, the B cell, has been identified by cell-type fractionation studies as a highly sensitive cellular target for the direct immunotoxic effects of TCDD (Dooley and Holsapple, 1988).

The actual molecular mechanism responsible for the immunotoxic effects produced by TCDD is unclear; however, many of the biological effects of HAHs are thought to be mediated by the AhR (Rowlands and Gustafsson, 1997). The AhR is a 95–110-kDa basic helix-loop-helix, ligand-dependent transcription factor (Burbach *et al.*, 1992) which, in the absence of ligand, is primarily located in the cytoplasm and is complexed with heat shock protein-90 and other partially characterized proteins (Pollenz *et al.*, 1994; Enan and Matsumura, 1996; Ma and Whitlock, 1997; Rowlands and Gustafsson, 1997). Ligand binding induces conformational changes in the AhR resulting in disassociation of the cytoplasmic complex and translocation of the liganded AhR into the nucleus (Pollenz *et al.*, 1994; Rowlands and Gustafsson,

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ABBREVIATIONS: HAH, halogenated aromatic hydrocarbon; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; DRE, dioxin-responsive enhancer; LD_{50}, 50% of lethal dose; RT, reverse transcriptase; PCR, polymerase chain reaction; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; BSAP, B cell-specific activator protein; PAGE, polyacrylamide gel electrophoresis; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

1997) where it forms a heterodimer with an 87-kDa basic helix-loop-helix protein called the ARNT protein (Reyes *et al.*, 1992). The AhR/ARNT complex can act as a transcription factor by binding specific DNA sequences termed DREs in the promoter regions of sensitive genes (Reyes *et al.*, 1992; Rowlands and Gustafsson, 1997). This mechanism has been primarily elucidated and characterized by studying HAH-induced up-regulation of drug metabolizing enzymes, such as CYP1A1, in hepatic tissue and hepatic cell lines. The involvement of an AhR-mediated mechanism in the toxicity, including immunotoxicity, induced by TCDD has not been clearly established. In fact, the most TCDD-susceptible species in terms of LD_{50}, the guinea pig, does not show a notable induction of liver microsomes with TCDD treatment (Kociba *et al.*, 1978). In contrast, the least TCDD-susceptible species in terms of LD_{50}, the hamster, shows a marked induction of liver microsomes with TCDD treatment (Matsumura, 1994). In addition to metabolic enzymes, other genes, such as those encoding plasminogen activator inhibitor-2, interleukin-1β, and transforming growth factor-α and -β, have been shown to be up-regulated or down-regulated with TCDD treatment (Sutter *et al.*, 1991; Gaido *et al.*, 1992) but the mechanism of this regulation is presently unknown.

Studies with congenic mice at the Ah locus and with Ah high responsive and Ah low responsive mouse strains, as well as structure-activity relationship studies have generally supported a role for the AhR in the immunotoxic effects produced by TCDD (Vecchi *et al.*, 1983; Davis and Safe, 1988; Kerkvliet *et al.*, 1990). However, in contrast to structure-activity relationships observed between AhR binding and immunotoxicity, the low affinity AhR ligand, 2,7-dichlorodibenzo-*p*-dioxin, and TCDD produced comparable inhibition of the T cell-dependent antibody forming cell response after subchronic treatment of mice *in vivo* and after direct addition to naive splenocytes *in vitro* (Holsapple *et al.*, 1986a, 1986b). The condition of TCDD exposure seems to be a factor in segregation of the immunotoxicity with the AhR in that, subchronic TCDD treatment produced a marked immunosuppression in DBA/2 (Ah low responsive), B6C3F1 (Ah high responsive), and congenic mice encoding for an AhR with low affinity for TCDD (Morris *et al.*, 1992; Holsapple *et al.*, 1986a). These results indicate a loss of the resistance originally seen in DBA/2 and congenic mice acutely treated with TCDD (Vecchi *et al.*, 1983; Kerkvliet *et al.*, 1990). In light of the above observations, it is unclear what role the AhR plays in mediating the toxicity of TCDD.

The objective of the current studies was to develop a cell line model system for the purpose of elucidating the role of the AhR in the well documented alteration of B cell function by TCDD. In this report, we describe two B cell lines that differ markedly in their expression of the AhR as well as in their sensitivity to TCDD. Furthermore, studies with these cell lines have identified the AhR as a critical factor in TCDD-induced inhibition of IgM secretion and have demonstrated an induction of AhR gene and protein expression after B cell activation with a differentiating signal.

Materials and Methods

Cell lines. The CH12.LX B cell line derived from the murine CH12 B cell lymphoma, which arose in B10.H-2^{a}H-4^{b}p/Wts mice (B10.A $\times$ B10.129), has been previously characterized (Bishop and Haughton, 1986) and was a generous gift from Dr. Geoffrey Haughton (University of North Carolina, Chapel Hill, NC). The BCL-1 B cell line was derived from a murine B cell lymphoma that spontaneously arose in a BALB/c mouse (Slavin and Strober, 1978). This cell line has been previously characterized (Gronowicz *et al.*, 1980) and was generously provided by Dr. Kathryn H. Brooks (Michigan State University, East Lansing, MI). CH12.LX and BCL-1 cell lines were grown in RPMI-1640 (Gibco BRL, Grand Island, NY) supplemented with heat-inactivated 10% bovine calf serum (Hyclone, Logan, UT), 13.5 mM HEPES, 23.8 mM sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, and 50 μM β-mercaptoethanol. The mouse hepatoma cell line, Hepa 1c1c7, was generously provided to our laboratory by Dr. Michael S. Denison (University of California, Davis, CA). Hepa 1c1c7 cells were cultured in α-minimal essential media (Gibco BRL, Grand Island, NY) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% bovine calf serum. All cells were maintained at 37° in an atmosphere of 5% CO_2.

Western blot analysis. Western blot analysis was performed on whole cell lysates from CH12.LX, BCL-1, and Hepa 1c1c7 cells. Cell lysates were prepared in buffer A (25 mM HEPES, 2 mM EDTA, 1 mM dithiothreitol, 10% glycerol and 20 mM sodium molybdate), homogenized, and centrifuged at 105,000 $\times$ *g* for 1 hr at 4°. Protein concentrations were determined by the Bradford protein assay (Sigma, St. Louis, MO). Cell lysate proteins were resolved by denaturing SDS-PAGE with 7.5% polyacrylamide (National Diagnostics, Atlanta, GA). The electrophoresed proteins were transferred to nitrocellulose (Amersham, Arlington Heights, IL). Protein blots were blocked in BLOTTO buffer (5% low-fat dry milk in 0.1% Tween 20, Tris-buffered saline) for 1–2 hr at 22°. Primary antibodies to the AhR (17-10B) and ARNT protein (20-9B), previously characterized by Pollenz *et al.* (1994), were a generous gift of Dr. Richard S. Pollenz (Medical University of South Carolina, Charleston, SC). Immunochemical staining was performed as previously described (Williams *et al.*, 1996) with the following exception. The anti-AhR antibody and the anti-ARNT antibody were diluted to 1 μg/ml in antibody dilution buffer (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.05% gelatin, 0.1% Nonidet P-40, and 0.5% bovine serum albumin in borate-buffered saline). Detection was performed using the electrochemiluminescence method (Amersham). Absorbance for the protein of interest was measured by densitometry using a model 700 imaging system (Bio-Rad, Hercules, CA).

Quantitative RT-PCR. Quantitative RT-PCR was performed as previously described (Williams *et al.*, 1996) with several modifications. Briefly, total RNA from each sample was isolated using Tri Reagent (Sigma). RNA samples were first analyzed for DNA contamination by PCR analysis without RT. RNA samples containing DNA were incubated with RNase-free DNase as previously described (Williams *et al.*, 1996). Total DNA-free RNA (100 ng) and internal standard (recombinant RNA) were reverse transcribed simultaneously in the same reaction tube. AhR and ARNT primers were as previously described (Williams *et al.*, 1996). Final reaction concentrations for the AhR PCR reaction were 4 mM $MgCl_2$ and 2.5 units of *Taq* DNA polymerase (Promega, Madison, WI). Samples were cycled 35 times with each cycle consisting of 94° for 15 sec, 59° for 30 sec, and 72° for 45 sec. The ARNT PCR was performed as described for the AhR PCR, except the samples were cycled 32 times. PCR products were visualized by ethidium bromide staining and quantitation was performed by assessing the absorbance for both the target and internal standard DNA using a Gel Doc 1000 video imaging system (Bio-Rad). The number of transcripts were calculated from a standard curve generated from the density ratio between the gene of interest and a specific internal standard concentration. Primers for the CYP1A1 gene were a generous gift of Dr. Dale Morris (J. D. Searle, Skokie, IL). The CYP1A1 PCR was performed as described above for the AhR PCR, except the annealing temperature was 56° and the samples were cycled 32 times.

ELISA. Supernatants were harvested from naive or LPS (30 μg/ml)-stimulated CH12.LX or BCL-1 cells after a 72-hr incubation at 37° in 5% CO_2 and were analyzed for IgM by sandwich ELISA. Briefly, 100 μl of supernatant or standard (mouse IgM, Sigma) were added to wells of a 96-well microtiter plate coated with anti-mouse immunoglobulin capture antibody (Boehringer Mannheim, Indianapolis, IN), and then incubated at 37° for 1.5 hr. After the incubation period, the plate was washed with 0.05% Tween-20 phosphate-buffered saline and H_20, followed by addition of a horseradish peroxidase anti-mouse IgM detection antibody (Sigma) and another incubation at 37° for 1.5 hr. Unbound detection antibody was washed from the plate after the incubation period with 0.05% Tween 20, phosphate-buffered saline, and H_2O. 2,2′-azinobis(3-ethylbenz thiazoline-sulfonic acid) ABTS substrate (Boehringer Mannheim) was added and colorimetric detection was performed over a 1 hr period using an EL808 automated microplate reader with a 405-nm filter (Bio-Tek, Winooski, VT). The DeltaSoft 3 computer analysis program (BioMetallics, Princeton, NJ) calculated the concentration of IgM in each sample from a standard curve generated from the absorbance readings of known IgM concentrations.

Statistical analysis of data. The mean ± standard error was determined for each treatment group of a given experiment. When significant differences occurred, treatment groups were compared with the vehicle controls using a Dunnett's two-tailed *t* test.

Results

AhR and ARNT expression in CH12.LX and BCL-1 B cell lines. Western analysis for AhR and ARNT was performed using whole cell lysates from the CH12.LX and BCL-1 B cell lines. Interestingly, an approximately 95-kDa AhR was markedly expressed in CH12.LX cells but was not detected in BCL-1 cells (Fig. 1A). The 87-kDa ARNT protein was well expressed in both B cell lines (Fig. 1B). To confirm a lack of AhR expression in BCL-1 cells, total RNA isolated from BCL-1 cells was analyzed by qualitative RT-PCR analysis, a more sensitive technique than Western analysis. In agreement with the above results, AhR transcripts were not detected in BCL-1 RNA (Fig. 2A). Quantitative RT-PCR analysis of basal AhR and ARNT transcripts demonstrated a much greater expression of ARNT in CH12.LX cells as compared with AhR (Fig. 2B). In addition, similar levels of ARNT transcripts were detected in BCL-1 cells as compared with CH12.LX cells (Fig. 2B).

AhR and ARNT regulate gene transcription in the CH12.LX B cell line. "Functionality" of AhR and ARNT was evaluated in CH12.LX cells by the ability of TCDD to induce CYP1A1 expression. Unlike the electrophoretic mobility sift assay, which is only an indicator of DRE binding, TCDD-induced up-regulation of *Cyp1a1* is an indicator of transcriptional regulation mediated by DRE binding, and thus is a more comprehensive indicator of AhR/ARNT function. Evaluation of *Cyp1a1* gene expression by quantitative RT-PCR demonstrated a marked and rapid increase in CYP1A1 transcripts after TCDD treatment. TCDD-induced CYP1A1 expression occurred as early as 2 hr and was maximal by 8 hr, remaining elevated throughout the time course (Fig. 3). Induction of *Cyp1a1* in CH12.LX cells is also dose-dependent. An 8-hr treatment of TCDD at concentrations of 0.003, 0.03, 0.3, and 3.0 nM induced CYP1A1 expression 14-, 38-, 44- and 120-fold, respectively, above the vehicle control. BCL-1 cells were also analyzed for *Cyp1a1* inducibility. As predicted because of the lack of AhR expression, CYP1A1 transcripts were not detected by qualitative RT-PCR analysis in TCDD-treated BCL-1 cells (Fig. 4).

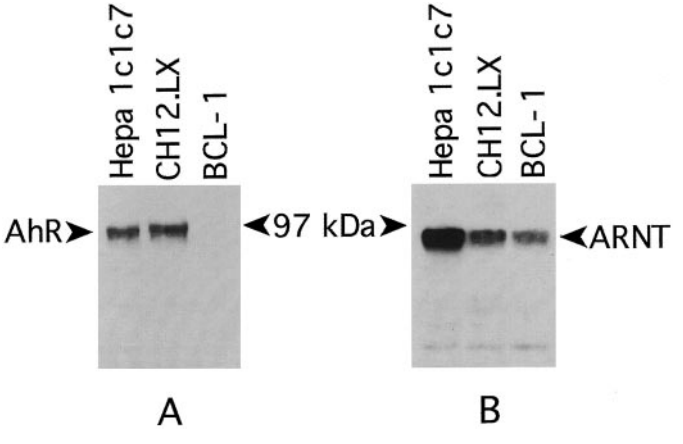

Fig. 1. Western blot analysis for the AhR and ARNT protein in the CH12.LX and BCL-1 cell lines. Whole cell lysate was isolated from untreated CH12.LX (5×10^5 cells/ml) and BCL-1 (5×10^5 cells/ml) cells. The Hepa 1c1c7 cells (5×10^5 cells/ml) served as a positive control. Cell lysate protein (70 μg) was loaded in each *lane*, resolved on a 7.5% SDS-PAGE gel, and probed with (A) 1 μg/ml anti-AhR antibody and (B) 1 μg/ml anti-ARNT antibody. Results are representative of more than two separate experiments.

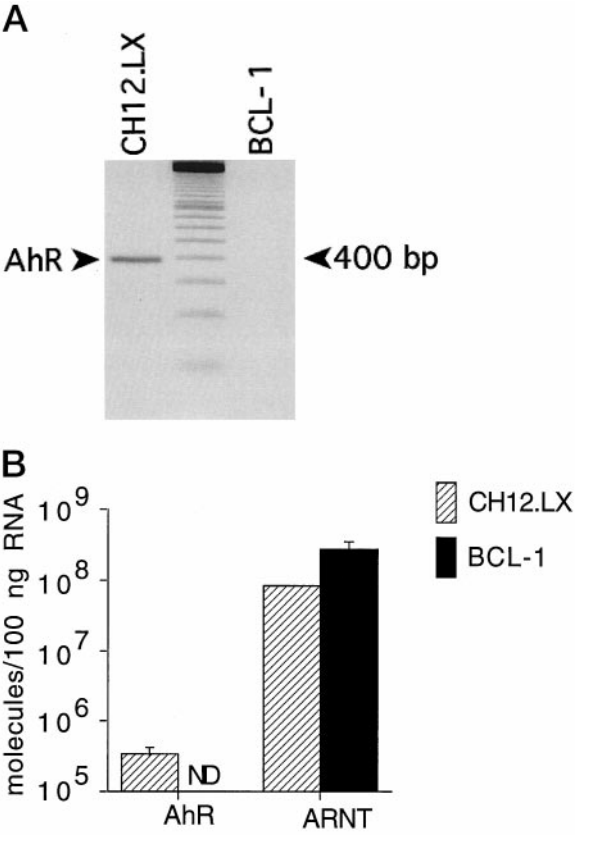

Fig. 2. Basal expression of AhR and ARNT transcripts in the CH12.LX and BCL-1 cell lines. Total RNA was extracted from untreated CH12.LX (5×10^5 cells/ml) and BCL-1 cells (5×10^5 cells/ml), and 100 ng of total RNA were analyzed by (A) qualitative RT-PCR analysis for AhR or (B) quantitative RT-PCR analysis for AhR and ARNT transcripts as specified under Experimental Procedures. AhR and ARNT mRNA transcripts are represented on the *y*-axis as molecules/100 ng of RNA. *ND*, samples in which transcripts were not detected; *bar*, mean ± standard error for two separate RNA isolations. The results are representative of more than two separate experiments.

TCDD alters immune function in CH12.LX B cells but not in AhR-deficient BCL-1 B cells. To assess the sensitivity of CH12.LX cells and BCL-1 cells to TCDD, LPS-induced IgM secretion was measured by ELISA. In primary B cells TCDD has been previously shown to suppress IgM secretory responses to the polyclonal B cell-activator LPS (Dooley and Holsapple, 1988) and to soluble anti-IgM (Karras and Holsapple, 1994). LPS can induce CH12.LX and BCL-1 cells to secrete IgM and treatment of CH12.LX cells with TCDD resulted in a marked inhibition of LPS-induced IgM secretion at doses as low as 0.03 nM TCDD (Fig. 5). In contrast, LPS-induced IgM secretion in AhR-deficient BCL-1 cells was not inhibited at concentrations as high as 3.0 nM TCDD (Fig. 6).

Differential expression of AhR in LPS-differentiated CH12.LX B cells. The effect of LPS-induced differentiation of the CH12.LX and BCL-1 cells on AhR gene expression was evaluated by RT-PCR. A marked up-regulation of AhR gene expression occurred by 4 hr in LPS-activated CH12.LX cells (Fig. 7). In contrast, LPS-induced activation of BCL-1 cells did not result in expression of the AhR gene (Fig. 8). Consistent with AhR gene expression, Western analysis for the AhR protein in LPS-activated CH12.LX cells demonstrated an increase in protein expression by 8 hr (Fig. 9). In addition, LPS treatment did not alter basal ARNT mRNA or protein expression (data not shown).

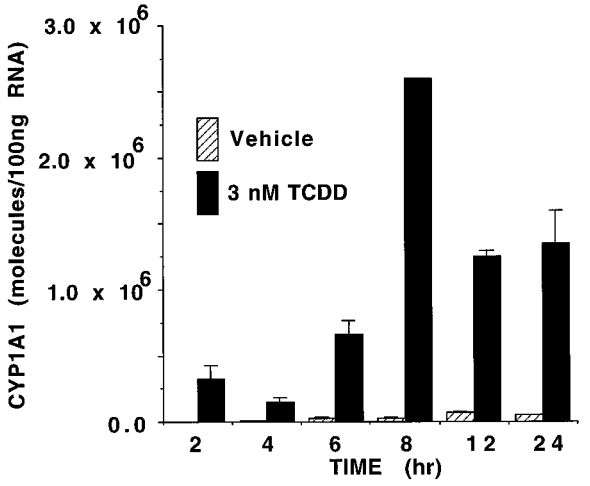

Fig. 3. Time course of TCDD-induced *Cyp1a1* induction in CH12.LX cells. CH12.LX cells (5×10^5 cells/ml) were treated with 3.0 nM TCDD or vehicle (*VH*, 0.01% DMSO) for selected time points. Quantitative RT-PCR analysis for CYP1A1 was performed on RNA extracted from each treatment group. CYP1A1 mRNA transcripts are represented on the *y*-axis as molecules/100 ng of RNA. *Bar*, mean ± standard error for two separate RNA isolations. The results are representative of more than two separate experiments.

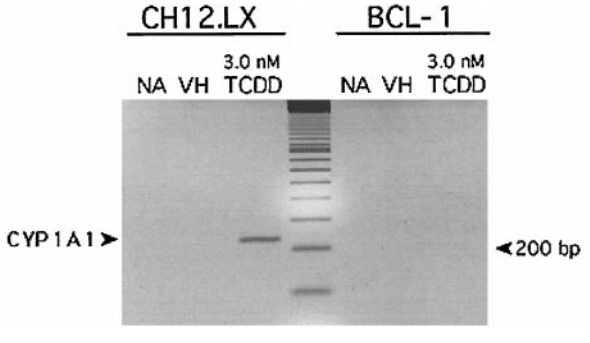

Fig. 4. Effect of TCDD on *Cyp1a1* induction in BCL-1 cells. BCL-1 (5×10^5 cells/ml) and CH12.LX (5×10^5 cells/ml) cells were treated with 3.0 nM TCDD or vehicle (*VH*, 0.01% DMSO) for 24 hr. Qualitative RT-PCR analysis for CYP1A1 was performed on RNA extracted from each treatment group. Results are representative of more than two separate experiments.

Discussion

We have identified two B cell lines that differ notably in their expression of the AhR as well as in their sensitivity to TCDD. The CH12.LX B cell line markedly expresses both the AhR and ARNT protein as determined by Western and RT-PCR analysis; whereas, the BCL-1 B cell line expresses only the ARNT protein and lacks expression of the AhR at the mRNA level. Although a basis for the loss of AhR expression in BCL-1 cells is unknown, it is noteworthy that the BCL-1 cell line is derived from an Ah high responsive BALB/c mouse strain, which has been characterized as expressing an AhR

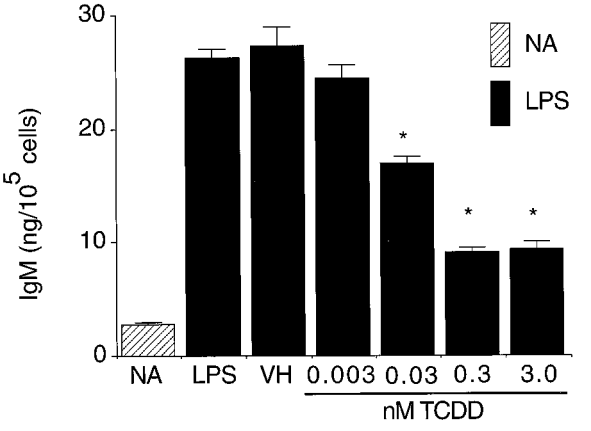

Fig. 5. Effect of TCDD on LPS-induced IgM secretion in CH12.LX. CH12.LX cells (1×10^4 cells/ml) were treated with LPS (30 μg/ml) and selected concentrations of TCDD or vehicle (*VH*, 0.01% DMSO). Supernatants were harvested at 72 hr and analyzed for IgM by sandwich ELISA as described under Experimental Procedures. Results from triplicate determinations are represented as mean IgM (nanograms/10^5 cells) ± standard error. *, values that are significantly different from the vehicle at $p < 0.05$. The results are representative of more than three separate experiments.

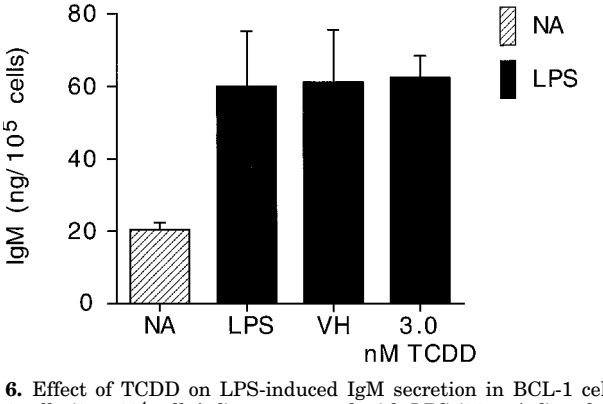

Fig. 6. Effect of TCDD on LPS-induced IgM secretion in BCL-1 cells. BCL-1 cells (2×10^4 cells/ml) were treated with LPS (30 μg/ml) and 3.0 nM TCDD or vehicle (*VH*, 0.01% DMSO). Supernatants were harvested at 72 hr and analyzed for IgM by sandwich ELISA as described under Experimental Procedures. Results from triplicate determinations are represented as mean IgM (nanograms/10^5 cells) ± standard error. *, values that are significantly different from the vehicle at $p < 0.05$. The results are representative of more than three separate experiments.

allele that encodes for an AhR with high affinity for TCDD (Poland and Glover, 1990). Further characterization of the AhR in the CH12.LX cells has verified a close similarity with results from studies conducted in primary lymphocytes. Specifically, the basal mRNA expression of AhR and ARNT in primary lymphocytes was very similar to that detected in CH12.LX cells (Williams *et al.*, 1996). In addition, both primary lymphocytes and CH12.LX cells exhibited a greater expression of ARNT transcripts as compared with AhR transcripts (Williams *et al.*, 1996). This difference in expression has been detected in a variety of tissues and has led to the speculation that ARNT has other biological roles (Carver *et al.*, 1994). As previously demonstrated in primary lymphocytes (Williams *et al.*, 1996; Crawford *et al.*, 1997), the AhR and ARNT protein were shown to be "functional" in the CH12.LX cells by their ability to regulate *Cyp1a1* transcription.

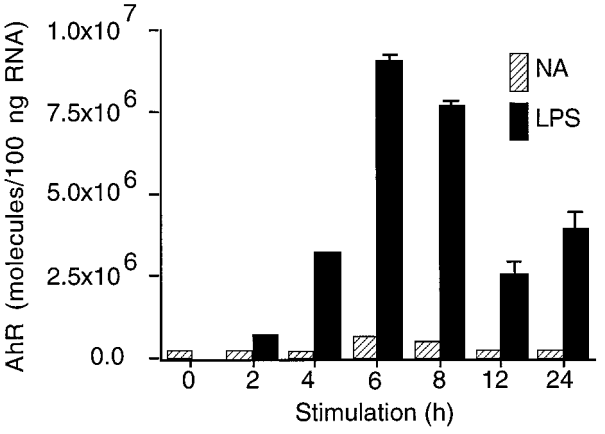

Fig. 7. Effect of LPS-induced differentiation on AhR gene expression in the CH12.LX cells. CH12.LX cells (5×10^5 cells/ml) were treated with LPS (30 μg/ml) for selected time points. Quantitative RT-PCR analysis for AhR was performed on RNA extracted from naive (*NA*) and LPS-stimulated cells at each time point. AhR mRNA transcripts are represented on the *y*-axis as molecules/100 ng of RNA. *Bars*, mean ± standard error for two separate RNA isolations. The results are representative of more than two separate experiments.

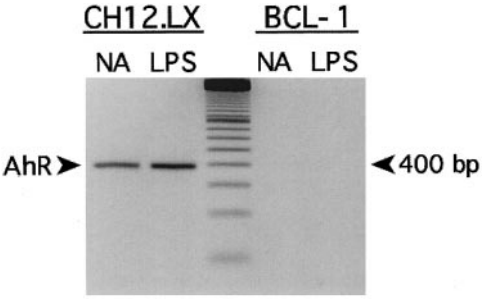

Fig. 8. Ahr gene expression in LPS-stimulated CH12.LX and BCL-1 cells. CH12.LX (5×10^5 cells/ml) and BCL-1 (5×10^5 cells/ml) cells were treated with LPS (30 μg/ml) for 6 hr. Qualitative RT-PCR analysis for AhR was performed on RNA extracted from naive (*NA*) and LPS-stimulated cells. Results are representative of more than two separate experiments.

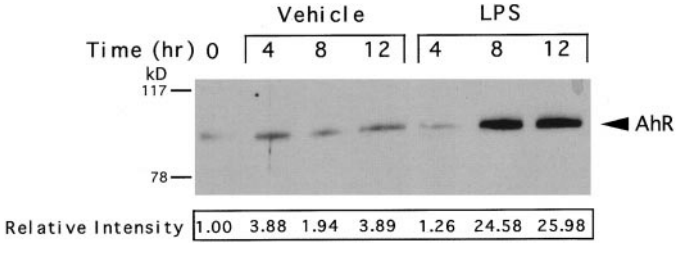

Fig. 9. AhR protein expression in LPS-stimulated CH12.LX cells. CH12.LX cells (1×10^5 cells/ml) were treated with LPS (30 μg/ml) for selected time points. Whole cell lysate was isolated from naive (*NA*) and LPS-stimulated cells at each *time point*. Cell lysate protein (12.5 μg) was loaded in each *lane*, resolved on a 7.5% SDS-PAGE gel and probed with 1 μg/ml anti-AhR antibody. Results are representative of more than two separate experiments.

As stated earlier, inhibition of IgM secretion from primary B cells is a sensitive biological consequence of TCDD exposure (Holsapple *et al.*, 1991); however, the involvement of the AhR in mediating this effect is unclear. In addition to the previously mentioned studies involving acute 2,7-dichlorodibenzo-*p*-dioxin treatment and subchronic TCDD treatment in which immunotoxicity did not segregate with the AhR (Holsapple *et al.*, 1986a, 1986b; Morris *et al.*, 1992), several other investigators have demonstrated TCDD-mediated events not dependent on a functional AhR. These include induction of *jun*B and c-*fos* (Puga *et al.*, 1992), induction of protein kinases (Bombick *et al.*, 1988), PLC activation (Beebe *et al.*, 1990), and Ca^{2+} influx (Puga *et al.*, 1992). Induction of immediate early genes such as *jun*B and c-*fos* are known to be involved in regulation of cellular proliferation and differentiation. In addition, increased protein phosphorylation that did not segregate with the AhR (Snyder *et al.*, 1993) and an increase in Ca^{2+} influx (Karras *et al.*, 1996) have been identified in primary B cells. The increase in Ca^{2+} influx may also be AhR-independent as demonstrated by Puga *et al.* (1992) in hepatoma cells. Moreover, increased protein phosphorylation (Snyder *et al.*, 1993) and Ca^{2+} influx (Karras *et al.*, 1996) were implicated in TCDD-induced suppression of the antibody response, further questioning the role of the AhR in suppression of antibody secretion.

Like primary B cells, CH12.LX cells respond to an LPS-differentiating signal with a significant increase in IgM secretion. Although lacking the AhR, BCL-1 cells are viable and are also capable of differentiating into antibody-secreting cells. Therefore, BCL-1 cells respond similarly to an LPS-differentiating signal as compared with CH12.LX cells, which suggests that these cells have all of the necessary signaling components for B cell activation. We have found that TCDD treatment of CH12.LX cells results in a sensitive and marked inhibition of IgM secretion similar to that seen in primary B cells; however, LPS-induced IgM secretion from the AhR-deficient BCL-1 cells is not sensitive to inhibition by TCDD. These results are the first to directly implicate a role for the AhR in mediating a TCDD-induced alteration of B cell function. It is important to emphasize that, although our conclusion is slightly tempered because of the fact that our results are obtained from cell lines originating from two different mouse strains, we believe that the cell lines are in fact quite comparable. As stated above, both lines are derived from Ah high responsive mouse strains as evidenced by sensitivity to TCDD and high affinity binding of TCDD to the AhR. Equally important, both cell lines respond similarly to an LPS-differentiating signal (i.e., secrete IgM).

A differential regulation of basal AhR gene expression was demonstrated in cells of different lineages using deletion constructs of the AhR 5′-flanking region (FitzGerald *et al.*, 1996). The authors concluded that this variation in regulation of AhR expression may provide the basis for differences

in the sensitivity of various tissues to TCDD (FitzGerald *et al.*, 1996). Seeming to be in contrast with the above conclusion, leukocytes are very sensitive to TCDD, yet they express relatively low levels of AhR as compared with other target organs, such as the liver (Williams *et al.*, 1996). However, an up-regulation of AhR gene and protein expression was recently demonstrated in PMA/Io-stimulated primary leukocytes revealing a possible explanation for the sensitivity of these cells to TCDD (Crawford *et al.*, 1997). In agreement with these results, an up-regulation of AhR gene and protein expression was detected in LPS-activated CH12.LX cells in the absence of TCDD. Taken together these results demonstrate an induction of the AhR upon lymphocyte activation and suggest a role for the AhR in cellular proliferation and/or differentiation. An additional consequence of AhR up-regulation may be an increased nuclear translocation and binding of ligand-activated AhR to DREs located in promoter regions of genes sensitive to TCDD. Masten and Shiverick (1995) recently identified negative regulation by TCDD of a B cell-specific gene. This seemed to be mediated by an AhR/DRE mechanism through a competition between the B cell-specific transcription factor, BSAP, and the AhR nuclear complex for binding to DNA that contained a DRE motif within the binding site for BSAP (Masten and Shiverick, 1995). It is likely that other genes involved in cellular proliferation and differentiation of B cells may also contain DREs within their promoter regions. If so, the activated AhR nuclear complex may directly interfere with DNA binding of lineage specific transcription factors, such as BSAP, or simply modulate gene expression by binding DREs. In addition to activated leukocytes, an up-regulation of AhR gene expression has been observed during monocyte differentiation (Hayashi *et al.*, 1995) and keratinocyte differentiation (Wanner *et al.*, 1995). The AhR has also been implicated in cell cycle regulation of Hepa 1c1c7 cells. Specifically, cells deficient in AhR have a longer doubling time then wild-type cells expressing the AhR. Introduction of antisense AhR cDNA into wild-type cells results in a longer doubling time resembling that of the AhR-deficient cells (Ma and Whitlock, 1996).

Until now, we have lacked an appropriate model to study the mechanisms involved in one of the most sensitive effects of TCDD exposure, alteration of B cell function. A majority of the work characterizing the AhR has been performed using the hepatic cell line model, Hepa 1c1c7, and various clones of this cell line that are defective in the AhR or ARNT protein (Miller *et al.*, 1983). However, B cells are unique among the cellular targets of TCDD in that these cells require cellular activation before mediating their effector functions. Because the AhR may have a significant role in cellular proliferation and/or differentiation as discussed earlier, the B cell may represent an important model for studying the role of the AhR in these processes.

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