2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN INDUCES ADSEVERIN GENE EXPRESSION IN MOUSE THYMUS - A THYMOCYTE SPECIFIC, AH-RECEPTOR MEDIATED EFFECT

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Introduction

The immune system is known to be sensitive to exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally related compounds, major effects being thymic atrophy and suppression of the immune response in experimental animals[1]. In the thymus, TCDD causes a reduction of mainly immature thymocytes, which partly is due to an initial inhibition of cell proliferation. However, while the proliferation returns to normal within a few days, the cell number in the thymus remains low for a long period of time[2]. It is clear that the thymic atrophy and most other TCDD induced toxic effects are mediated via the Ah-receptor (AhR), a ligand activated transcription factor. Several Ah-receptor regulated genes, of which CYP 1A1 is the most prominent, have also been identified[3,4]. However, none of these earlier described genes can fully explain the toxicity caused by TCDD.

We have previously found (communicated at Dioxin 1998 and Eurotox 1999, manuscript submitted) that TCDD increases the mRNA levels of adseverin, an actin binding protein in mouse thymus. Adseverin is involved in actin redistribution[5], and thus is important for normal lymphocyte activities such as migration and activation[6]. The induction of adseverin is restricted to the adult and fetal thymus, fetal liver and adult spleen and thus it seems to be immune specific. The induction can be observed as early as 3h after TCDD exposure (earliest time point examined), before any reduction in cell number is detected, suggesting that the induction is directly regulated by TCDD.

By using radiation chimeras in which the AhR is lacking in either the hematopoietic compartment or in epithelial cells, Staples and coworkers recently showed that the thymic atrophy induced by TCDD is due to a direct effect on the hematopoietic compartment in thymus[7].
In this study we have used these chimeras to investigate if the increase in adseverin mRNA levels is a direct effect of TCDD on thymocytes or an indirect effect via thymic epithelium. We have also compared the inducibility of adseverin, upon TCDD treatment, in thymocytes versus epithelial cells by the use of fetal thymus organ cultures (FTOC).

Materials and Methods

Aryl hydrocarbon receptor (AhR) knockout mouse chimeras were produced by hematopoietic reconstitution of irradiated mice as described previously[7]. The different chimeric mice constructed were (1) $AhR^{+/+}$ bone marrow donor into $AhR^{+/+}$ background recipient (+/+→+/+), (2) $AhR^{+/+}$ bone marrow donor into $AhR^{-/-}$ recipient (+/+→-/-), (3) $AhR^{-/-}$ bone marrow donor into $AhR^{+/+}$ recipient (-/-→+/+) and (4) $AhR^{-/-}$ bone marrow donor into $AhR^{-/-}$ recipient (-/-→-/-). In addition, knockout animals (AhR-/-) were included as nonchimeric controls. At eight weeks of age, i.e. after four weeks of reconstitution, male mice were randomly divided into treatment groups containing three to five animals that received vehicle (olive oil) or 30 µgTCDD/kg i.p. The animals were killed 10 days after the treatment and the thymocytes were isolated and subjected to

RNA isolation with TRIzol reagent (Life Technologies) according to the manufacturer's instructions.

C57BL/6 mice used for FTOCs were bred in our own facility. Original breeding pairs were obtained from B&K Universal, Solna Sweden. FTOCs were prepared as described previously[2]. Briefly, pregnant C57 BL/6 mice were killed on gestational day 15 (day of plug=day 0) by cervical dislocation and thymic lobes from their fetuses were collected. The thymic lobes were placed on filters, resting on metallic grids in 2,5 ml culture medium (RPMI 1640 supplemented with 10% fetal calf serum, 2mM L-glutamine, 100 U/ml penicillin and 0,1 mg/ml streptomycin). The thymic lobes were cultured with or without 135mM 2-deoxyguanosine at 37°C and 5 %CO_2 in a water-saturated atmosphere. At day 4 the cultures were fed by transferring the filter inserts to new culture disks containing fresh media with or without deoxyguanosine. These groups were then divided into a control and a TCDD treated group and TCDD or solvent (1,4-dioxan), were added to a concentration of 10 nM. Twenty-four hours after addition of TCDD/solvent to the lobes, the lobes were harvested. Thymocytes were isolated from half of the non-deoxyguanidin treated cultures and thymocytes, whole thymus and thymic stroma were directly subjected to RNA isolation with TRIzol.

Reverse transcription-polymerase chain reactions (RT-PCR) on RNA from chimeras and FTOCs were performed with adseverin and CYP 1A1 specific primers according to a modified protocol published by Lai and coworkers[8]. The PCR products were separated on an EtBr containing agarose gel and photographed on a GDP 5000 Gel documentation system from UVP, Cambridge, England. The expression of adseverin and CYP 1A1 was calculated with the housekeeping gene, hypoxanthine phosphoribosyltransferase as an internal standard.

Results and discussion

Using RT-PCR we measured the mRNA levels of adseverin in thymocytes isolated from the different chimeras/AhR knockouts treated with TCDD (30μg/kg) or vehicle. We found a TCDD induced increase in the adseverin mRNA levels in thymocytes with $AhR^{+/+}$ phenotype, irrespective of the phenotype of the epithelial cells. No induction was observed in $AhR^{-/-}$ thymocytes. The induction of CYP 1A1 gene expression, another Ah-receptor responding gene, followed the same pattern (Table 1). This shows that the increase of adseverin gene expression is dependent on the presence of AhR in the hematopoietic cells and that the induction of adseverin is not mediated via the thymic epithelium.

Table 1. Effects of TCDD treatment of AhR radiation chimeras

	+/+→+/+	+/+→-/-	-/- → +/+	-/- → -/-	AhR -/-
Adseverin induction	+	+	-	-	-
CYP1A1 induction	+	+	-	-	-
Thymus Atrophy (See reference 7)	+	+	-	-	-

Furthermore, the results from the RT-PCR on FTOC samples (Table 2) shows that TCDD induces an increase in adseverin mRNA levels in thymocytes but does not affect the level of adseverin in deoxyguanidin treated fetal thymic lobes i. e. thymic epithelial cells. This is in contrast to Cyp 1A1 gene expression, which is induced at the mRNA level both in thymocytes and stroma.

Table 2. Induction of Adseverin and CYP 1A1 in different thymic compartments after TCDD treatment of FTOCs

	Whole thymus	Thymic stroma	Isolated thymocytes
Adseverin induction	+	-	+
CYP 1A1 induction	+	+	+

Our results indicate that the TCDD induction of adseverin is AhR-dependent and that it is the hematopoietic cells in the thymus that are the target cells. This is also in agreement with the earlier finding of Staples and coworkers[7]. Considering the physiological role of adseverin, the induction might play a role in the immunotoxicity caused by TCDD.

References

1. Kerkvliet, N.I. (1995) Environ Health Perspect. 103 (Suppl. 9), 47
2. Lundberg K., Grönvik K-O., Goldschmidt T.J., Klareskog L and Dencker L. (1990) Chem.-Biol Interactions, 74, 179
3. Okey A.B., Riddick D.S., Harper P.A. (1994) Toxicol Lett. 70, 1
4. Schmidt J.V. and Bradfield C.A. (1996) Ann Rev Cell Dev Biol. 12, 55
5. Rodrígues Del Castillo A., Vitale M.L. and Trifaró J.-M. (1992) J. of Cell Biol. 119, 797
6. Serrador J.M., Nieto M. and Sánchez-Madrid F. (1999) Trends Cell Biol. 9, 228
7. Staples J.E., Murante F.G., Fiore N.C., Gasiewicz T.A. and Silverstone, A.E. (1998) J Immunol. 160, 3844
8. Lai Z.-W., Fiore N.C., Gasiewicz T.A. and Silverstone A.E. (1998) Toxicol Appl Pharmacol. 149, 167