PRIMARY LYMPHOCYTES HAVE A FUNCTIONAL ARYL HYDROCARBON RECEPTOR

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread environmental pollutant that is highly toxic to humans and animals. It has been reported that most of the toxic effects of TCDD result from the alteration of gene expressions through the aryl hydrocarbon receptor (AhR), which is a ligand-activated transcription factor[1,2]. In the absence of a ligand, AhR localizes in cytosol with Hsp90. When it binds a ligand, such as TCDD, it translocalizes in the nucleus and forms a heterodimer with the AhR nuclear translocator (ARNT). This heterodimer activates transcription through binding to a xenobiotic response element (XRE) that is present in the upstream regulatory region of target genes. It is established that TCDD induces many genes, including cytokines, oncogenes, and drug-metabolizing enzymes. Especially, the cytochrome P450 1A1 (CYP1A1) gene is a highly sensitive target of TCDD.

TCDD induces immunosuppression, affecting both humoral and cellular immune responses[3-5]. With regard to mature lymphocytes, B cell lines have been shown to possess the functional AhR and express the CYP1A1 gene when exposed to TCDD[6]. On the other hand, it has been reported that TCDD cannot induce CYP1A1 in T cell lines because of the lack of AhR or inability of AhR for transcriptional activation[7,8], which suggests that TCDD indirectly affects T cell-mediated immune responses. Since the responsiveness of primary mature T lymphocytes has not been investigated, we here studied whether spleen B and T cells of mice have functional AhR and respond to TCDD in primary culture and *in vivo*.

Materials and Methods

Separation of T and B cells from splenocytes

T and B cells were separated from splenocytes with a MiniMACS separation system (Miltenyi Biotec, CA, USA) according to the manufacturer's instructions. Briefly, splenocytes were bound to MACS CD45R (B220) MicroBeads (for B cells) or biotinylated anti-mouse CD3ε monoclonal antibody with MACS Streptavidin MicroBeads (for T cells). The magnetically labeled splenocytes were separated on a column, which was placed in the magnetic field of a MACS separator. After removal of the column from the magnetic field, the magnetically retained cells were eluted as positively selected cells.

Primary cell culture

Cells were maintained in RPMI 1640 supplemented with 10 mM HEPES (pH 7.1) buffer, containing 0.5 mM 2-mercaptetanol, 1 mM sodium pyruvate, 10% FCS, 100 units/ml penicillin and 100 μg/ml streptomycin. TCDD dissolved in DMSO was added to the cells at a final concentration of 10 nM. After splenic T and B cells were cultured for 4, 8, and 24 hours, total RNA was extracted from these cells as described above.

Animal treatment

Female C57BL/6N mice were purchased from CLEA Japan. Inc (Tokyo, Japan) and subjected to acclimatization for 3 days before use. TCDD was dissolved in nonane and diluted with corn oil. C57BL/6N mice were injected with a single dose of 20 μg/kg body weight. After 3 and 5 days, mice were killed, and total RNA was extracted from spleen T and B cells using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The animals were handled throughout this study according to the guidelines for animal care of NIES.

Semiquantitative RT-PCR

Semiquantitative RT-PCR was performed using an RNA LA PCR^{TM} kit (AMV) ver1.1 (TaKaRa Biomedicals, Kyoto, Japan). Total RNA was used for cDNA synthesis with reverse transcriptase. Hypoxanthine phosphoribosyltransferase (HPRT), a housekeeping gene, was amplified together with the cDNA of AhR, ARNT, or CYP1A1 in the same reaction tube. To analyze the amplification of both cDNAs in the linear range, the primer drop method was used to calibrate for the different expression levels of HPRT versus AhR, ARNT, or CYP1A1. PCR products were separated on 1.2% agarose gels and analyzed by Scientific Imaging Systems (Kodak, NY, USA).

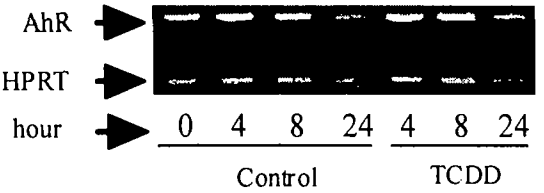
Results and Discussion

We investigated the responsiveness of primary lymphocytes to TCDD. First, we examined the AhR and ARNT gene expressions in the lymphocytes of C57BL/6N mice. Semiquantitative RT-PCR revealed that similar amounts of these genes were expressed in spleen T cells, as well as spleen B cells and splenocytes (Fig. 1). In addition, the expressions of AhR and ARNT genes were not affected by TCDD during culture for up to 24 hours.

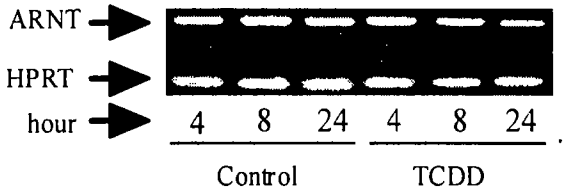
Next, we examined CYP1A1 mRNA induction by TCDD in these lymphocytes to determine whether the expressed AhR and ARNT were functional or not. As suggested by the previous reports[6], CYP1A1 gene was induced in spleen B cells by the addition of TCDD to the primary culture. Although it has been reported that TCDD did not induce CYP1A1 in T cell lines, the present results showed that TCDD induced CYP1A1 mRNA in spleen T cells as it did in spleen B cells. Besides, the kinetics of the induction until 24 hours was similar in these cells. Furthermore, the induction of CYP1A1 in spleen T cells was also observed when exposed to TCDD *in vivo*.

These results indicate that spleen T cells, as well as spleen B cells, express functional AhR and can directly respond to TCDD. Our recent results revealed that TCDD exposure *in vivo* affected cytokine production by spleen T cells. TCDD might induce these effects through the direct activation of AhR in T cells.

(A) AhR



(B) ARNT



(C)

Time (hour)	0	4	8	24
AhR control	1.47	1.75	2.22	1.38
AhR TCDD	—	1.94	1.8	1.25
ARNT control	—	0.94	0.83	0.77
ARNT TCDD	—	0.84	0.91	0.91

Fig.1. (A), (B) RT-PCR analysis of AhR and ARNT mRNA in splenocytes from mice cultured with 10 nM TCDD. Total RNA was prepared, reverse-transcribed and amplified by PCR for AhR and ARNT in conjunction with HPRT mRNA as an internal control. (C) Expression of AhR and ARNT was quantified by densitometric scanning of gel images and normalized to HPRT mRNA. The results shown were representative of two separate experiments.

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