TCDD-Induced Transcriptional Profiles in Different Mouse Strains that Have an Identical AhR Genotype

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an environmental contaminant that is known to cause hepatotoxicity, teratogenicity and carcinogenicity. A characteristic feature in the toxicity of TCDD is exceptionally large differences in susceptibility among animal species or even strains belonging to the same species. Among inbred mouse strains, C57BL/6 is the most TCDD sensitive strain so far reported while DBA/2 is known to be less sensitive. The DBA/2 strain requires a 10-20 times higher TCDD dose to manifest toxicity than does the C57BL/6 strain[1]. A much greater difference (about 1000-fold) in susceptibility to the acute lethality of TCDD exists between two rat strains, Long-Evans (Turku AB; L-E) and Han/Wister (Kuopio, H/W) [2, 3]. These strain differences in susceptibility to TCDD have now been elucidated to be due to the difference in ligand binding affinity or transcriptional activity of the aryl hydrocarbon receptor (AhR). Actually the C57BL/6 type AhR (AhR^b) showed 6-fold higher ligand binding affinity than the DBA/2 type AhR (AhR^d) [4]. The H/W rat AhR has a C-terminal truncation of the transactivating domain compared to the L-E rat AhR [5].

On the other hand, there is considerable species variability in response sensitivity to TCDD that cannot be ascribed simply to polymorphisms of the AhR gene. A non-AhR gene susceptibility loci for hepatic porphyria has been observed in mice treated with iron compounds prior to TCDD injection by using a quantitative trait locus analysis of an F2 intercross between susceptible C57BL/6 and resistant DBA/2 stains [6]. In the rat, a gene B with Han/Wistar type AhR is likely to be involved in resistance to TCDD lethality [7]. These observations suggest that other modulating genes, so-called "modifier genes", have profound effects on the AhR-mediated gene expression phenotype.

Based on the nucleotide sequence of the AhR coding region, the BALB/c, CBA/J, and C3H/He mouse strains are clustered together on a single branch [8]. In the present study, we try to

confirm the existence of modifiers by using microarray analysis to examine hepatic gene expression after TCDD exposure in BALB/c, CBA/J, and C3H/He mice. To recognize the existence of a modifier besides the AhR, it is a prerequisite experimental condition that the analyzed strains have an identical AhR genotype. Therefore, we selected BALB/c, CBA/J, and C3H/He mice as the model animal. Gene expression microarray analysis can monitor expression from different strains on a genome-wide scale. This experimental approach may eventually allow recognition of the various regulators in different strains after TCDD exposure. The aim of the present study is to compare hepatic gene expression patterns in different strains of mice with an identical AhR nucleotide sequence, and to detect good maker genes in order to confirm the existence of modifiers.

Methods and Materials

Mice: Animals were treated in a humane manner according to the guidelines for animal experiments at the National Institute for Environmental Studies. Female mice of the BALB/c, CBA/J, and C3H/He strain, 7 weeks old, were purchased from Charles River Inc. (Tokyo, Japan). The animals were provided food and water *ad libitum* and kept on a 12-h light/12-h dark cycle. We sequenced the AhR coding region of BALB/c, CBA/J, and C3H/He mice, and confirmed that the nucleotide sequence of the AhR coding region in the all of three mouse strains was identical.

TCDD exposure: TCDD (>99.5% pure) was purchased from Cambridge Isotope Laboratory (Andover, USA). TCDD was dissolved in n-Nonane (Nacalai Tesque, Japan), followed by further dilution in corn oil. For vehicle treatment, we used n-Nonane diluted with corn oil as prepared for the TCDD solution. Groups of three mice were given a single oral dose of TCDD (0.4, 4, and 40 μg/kg), or an equivalent volume of vehicle. After 24 h exposure, livers were dissected and were immediately frozen in liquid nitrogen. The samples were kept at -80 ^{o}C until RNA extraction.

Samples preparation, hybridization and array analysis: Total RNA was extracted by RNeasy Mini Kit (QIAGEN, Valencia, USA), cDNA synthesis, biotin-label cRNA synthesis and array analysis (Affymetrix [9]) were performed according to the suppliers' protocols. The Affymetrix GeneChip Mouse Expression Array 430A was used for the experiment. The GeneChip Operating Software (GCOS) (Affymetrix) was used to perform gene expression analysis.

The changes in gene expression levels observed in TCDD-exposed liver tissues at graded TCDD doses were compared to those of vehicle controls, by using data from two independent experiments in which total hepatic RNA extracted from 3 mice was mixed and used as one array sample.

Results and Discussion

TCDD-induced alterations in gene expressions common to the three strains of mice

When we examined the altered expression levels of genes in the liver from female BALB/c, CBA/J, and C3H/He mouse strains, we found that out of 22,690 transcripts, 26 genes were up-regulated, and 4 genes were down-regulated by TCDD in all three strains of mice. All these 30 genes were found to have a 2-fold or greater change in expression when compared to the vehicle controls in two independent experiments. Gene classification showed that these are genes responsible for response stimulus, metabolism, transcription regulation, cell signal cascade, and cell differentiation and growth. A battery of AhR-dependent genes such as CYP1A1, CYP1A2 and CYP1B1, well-established TCDD inducible genes, were up-regulated by TCDD. This result, taken as a positive control, suggests the validity of the microarray analysis in the present study. Diagrams showing the number of up-regulated and down-regulated genes in each mouse strain are presented in Figure 1.

TCDD-induced alterations in gene expression in BALB/c strain only

In order to find unique genes that are either up- or down-regulated by TCDD, we selected genes for which the expression level was changed 2-fold or greater in one strain, but not in the other two strains. There were 3 up-regulated genes and 18 down-regulated genes that were found in the BALB/c strain only. No genes, were significantly affected by TCDD only in the C3H/He or CBA/J strains (Fig 1).

Among the 21 genes that were affected by TCDD exposure only in the BALB/c strain, we focused on the glutathione S-transferase mu 6 (GSTm6) gene in the present study. Cytosolic glutathione S-transferases (GSTs) that are classified by four classes: alpha, mu, pi, and theta [10], are an important family of multifunctional isoenzymes that play a role in the protection of tissues by the detoxification of hazardous and carcinogenic compounds. In the vehicle-treated control liver tissues from the three strains of mice, constitutive expression levels of GSTm6 were nearly identical, but not in TCDD exposed liver tissues. The expression level of GSTm6 was dose-dependently increased in the BALB/c strain only, while it was not changed at all in the C3H/He and CBA/J strain mice.

In the present study, no change in the expression levels of AhR and AhR nuclear translocator (Arnt) in the liver specimens was found among the three different mouse strains that were administered graded TCDD doses. This result suggests that the difference in the induced level of GSTm6 between BALB/c and the other two strains was not due to different transcription levels of AhR and Arnt. Moreover, CYP1A1 expression levels were dose-dependently induced similarly in the three strains, indicating that the induction of GSTm6 in BALB/c strain was not due to a difference in the extent of ligand binding of AhR. Since basal expression levels of GSTm6 levels were similar among the three strains of mice without TCDD administration, significant induction of Gstm6 in BALB/c only by TCDD was not due to a difference in constitutive expression of GSTm6. Since the amino acid sequence of the AhR was identical among the three strains, the induction of GSTm6 induction by TCDD only in BALB/c mice strongly supports the notion that, this TCDD response requires modifier genes of AhR activity. GSTm6 is a good marker gene for detecting the existence of modifiers. Further research is necessary to identify the modifier genes that appear to regulate the induction of GSTm6 along with the AhR.

In conclusion, the present study analyzed and compared the gene expression profiles after TCDD exposure in different mouse strains with the same AhR amino acid sequence, and confirmed some unique alterations in gene expression in the BALB/c strain. The results provide

fundamental information that will be necessary to identify the modifier gene(s) responding to TCDD, besides AhR, in some strains of mice.

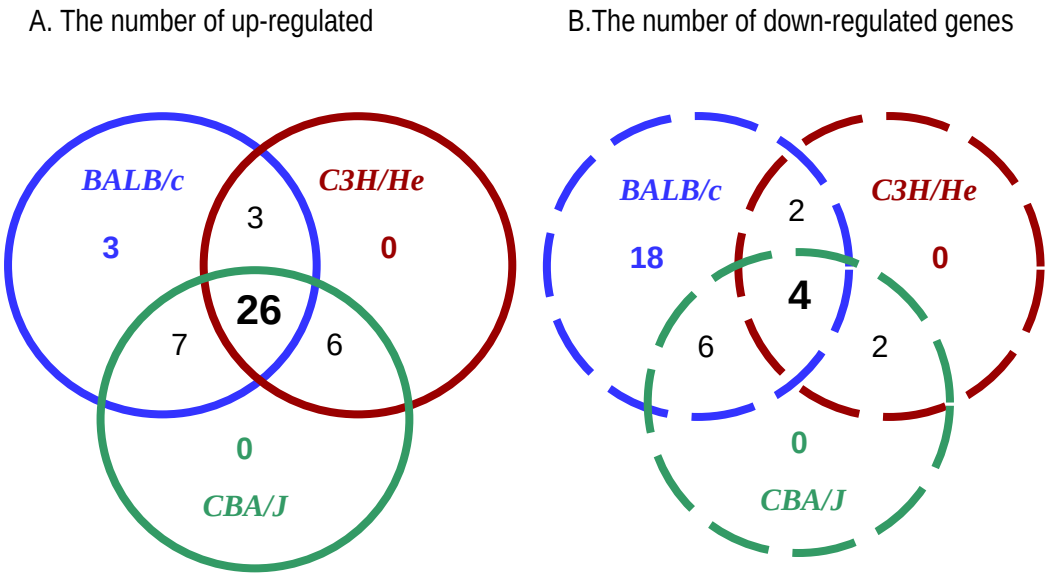

Fig.1 The number of up- and down- regulated genes in each mouse strain.

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