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# Role of aryl hydrocarbon receptor polymorphisms on TCDD-mediated CYP1B1 induction and IgM suppression by human B cells

Natalia Kovalova [a,b], Maria Manzan [b], Robert Crawford [b], Norbert Kaminski [a,b,*]

[a] Department of Pharmacology and Toxicology, Michigan State University, Lansing, MI 48824, USA
[b] Institute for Integrative Toxicology, Michigan State University, Lansing, MI 48824, USA

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ABSTRACT

Previous studies have demonstrated that most of the intraspecies variation in sensitivity to the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), including suppression of antibody responses, in murine models is due to single nucleotide polymorphisms (SNPs) within the aryl hydrocarbon receptor (AhR) gene. The underlying reason for variation in sensitivity to TCDD-induced suppression of IgM responses among humans is not well understood, but is thought, in part, to be a result of different polymorphic forms of the AhR expressed by different individuals. In this study, the functional properties of six (P517S, R554K, V570I, V570I + P517S, R554K + V570I and P517S + R554K + V570I) human AhR variants were examined in the human B cell line, SKW 6.4. TCDD-induced Cyp1B1 and Cyp1A2 mRNA expression levels and Cyp1B1-regulated reporter gene activity, used for comparative purposes, were markedly lower in SKW cells containing the R554K SNP than in SKW-AHR$^{+}$ (control AhR) cells. Furthermore, all AhR variants were able to mediate TCDD-induced suppression of the IgM response; however, a combined P517S + R554K + V570I variant partially reduced sensitivity to TCDD-mediated suppression of IgM secretion. Collectively, our findings show that the R554K human AhR SNP alone altered sensitivity of human B cells to TCDD-mediated induction of Cyp1B1 and Cyp1A2. By contrast, attenuation of TCDD-induced IgM suppression required a combination of all three SNPs P517S, R554K, and V570I.



## 1. Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a physicochemically stable, highly lipophilic environmental contaminant, generated mostly as a by-product of industrial processes and waste incineration. Biological and toxic effects of TCDD include induction of drug metabolizing enzymes, immune suppression, wasting syndrome and endocrine disruption (Pohjanvirta and Tuomisto, 1994; Poland and Knutson, 1982). Suppression of the immune system had been described in many animal species and is one of the most sensitive consequences of TCDD exposure (Holsapple et al., 1991; Sulentic and Kaminski, 2011). Moreover, TCDD had been shown to directly and specifically target B cell function (Dooley and Holsapple, 1988).

Studies with AhR-deficient experimental models, including mice, demonstrated that most, if not all, of the toxic effects of dioxins are mediated by the aryl hydrocarbon receptor (AhR) (Harrill et al., 2015; Mimura and Fujii-Kuriyama, 2003). In the absence of ligand binding, AhR is localized to the cytosol in a complex with heat shock protein 90 (HSP90), X-associated protein 2 (XAP2), and telomerase binding protein (p23) (Carver et al., 1998; LaPres et al., 2000). Upon ligand binding, the AhR translocates into the nucleus, where it heterodimerizes with AhR nuclear translocator (Arnt). AhR-Arnt heterodimers can bind dioxin responsive elements (DREs) located in the promoter region of TCDD responsive genes to influence transcription (Hankinson, 1995; Morel and Barouki, 1998; Senft et al., 2002).

The AhR signaling pathway is conserved broadly across species (Hahn, 2002); however, remarkable differences in species, strain and gender sensitivity to TCDD-mediated biological and toxicological effects exist (Bello et al., 2001; Enan et al., 1996; Kleeman et al., 1988). For example, the lethal dose 50% (LD50) for TCDD vary from 1 μg/kg for guinea pig, the most sensitive animal species, to >5000 μg/kg for hamster, the most resistant (Poland and Knutson, 1982). Similarly, AhR affinity and sensitivity to TCDD is about 10-fold higher in mice expressing the AhR$^{b1}$ allele as compared to mice that express the AhR$^{d}$ allele (Poland et al., 1994). The molecular basis for decreased affinity of the product of the AhR$^{d}$ allele for TCDD is a single nucleotide polymorphism (SNP) at the codon 375, which causes valine for alanine substitution in the ligand-binding domain (Poland and Glover, 1990).

Differences in sensitivity to TCDD-induced biological responses also occur in humans including Cyp1B1 induction and suppression of the IgM response (Catteau et al., 1995; Harper et al., 2002; Lu et al., 2010). These differences in human responsiveness to TCDD are likely due, in

* Corresponding author at: Institute for Integrative Toxicology, Michigan State University, 1129 Farm Lane, Room 315, Food Safety and Toxicology Building, East Lansing, MI 48824, USA.
*E-mail addresses:* kovalova@msu.edu (N. Kovalova), ale.manzan@gmail.com (M. Manzan), crawfo28@msu.edu (R. Crawford), kamins11@msu.edu (N. Kaminski).

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part, to SNPs within the AhR. Most of the known human AhR SNPs occur within exon 10, a region that encodes the transactivation domain of the receptor. Experimental evidence suggests that SNPs in the transactivation domain may result in differentially recruited coactivator/corepressor complexes, and consequently differentially regulate gene expression (Flaveny et al., 2008; Flaveny et al., 2010). Indeed, previous studies characterizing polymorphisms at the codon 517, 554 and 570 led to complicated and conflicting interpretations of the functional effects of these polymorphisms (Wong et al., 2001b; Celius and Matthews, 2010). For instance, the codon 554 variant correlated with induced Cyp1A1 activity in one study (Smart and Daly, 2000) but not in the others (Cauchi et al., 2001; Kawajiri et al., 1995). The combined occurrence of polymorphisms at codons 554 and 570, or 554 and 517 produced an AhR that failed to induce Cyp1A1 mRNA expression (Wong et al., 2001b). Since these studies were performed in mouse hepatoma cells, it is unknown whether a similar observation would be made in human B cells.

The objective of the current studies was to investigate the influence of identified human AhR polymorphisms on the ability of the receptor to mediate well-established TCDD-induced biological responses including induction of drug metabolizing enzymes and suppression of the IgM response in human B cells. Here we describe SKW 6.4 B cell lines that were stably transduced to express either a control AhR (SKW-AHR$^+$) or one of six different polymorphic forms of the human AhR (P517S, R554K, V570I, V570I + P517S, V570I + R554K, and V570I + R554K + P517S). The characterization of these polymorphisms within a specific cellular context provides new insights into the effects of structural variations in the human AhR on functional outcomes.

## 2. Materials and methods

### 2.1. Chemicals and reagents

TCDD in dimethyl sulfoxide (DMSO) (purity 99.1%) was purchased from AccuStandard Inc. (New Haven, CT). DMSO and lipopolysaccharide (LPS) (*Escherichia coli*, catalog no. L2755-10MG) were purchased from Sigma-Aldrich (St. Louis, MO). The anti-human AhR antibody was purchased from eBioscience (catalog no. 14–9854-82, San Diego, CA). The anti-mouse immunoglobulin capture antibody and the horseradish peroxidase anti-mouse IgM detection antibody were purchased from Boehringer Mannheim (Indianapolis, IN) and Sigma-Aldrich, respectively. The pokeweed mitogen (PWM) and puromycin were purchased from Sigma-Aldrich (St. Louis, MO, Lot. O L8777-5MG and P8833).

### 2.2. Cell lines

The SKW 6.4 cell line has been previously characterized by Ralph et al. (1984) and was obtained from ATCC (American Type Culture Collection, Rockville, MD). Cells were maintained under standard conditions (5% CO2/95% air, 98% humidity, 37 °C) in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% bovine calf serum (HyClone Laboratories, Logan, UT), 13.5 mM HEPES, 23.8 mM sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate and 2.5 g/l dextrose. Cells were cultured in suspension in 25-cm$^2$ flasks (Becton Dickinson, Plymouth, GB) at density of $<10^5$ cells/ml.

HEK293T cells were used in the co-culture experiments to generate SKW-based cell lines that stably express different polymorphic forms of the AhR and during transient transfections with Cyp1B1-regulated luciferase reporter constructs. HEK293T cells were cultured in DMEM supplemented with 10% bovine calf serum (HyClone Laboratories, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate. Cells were kept in a thermoregulated incubator at 37 °C, 5% $CO_2$ and had 80% confluence when used.

### 2.3. Animals

Specific pathogen-free, female C57BL/6 mice and Sprague-Dawley rats (5–8 weeks of age) were purchased from Charles River (Portage, MI). Animals were randomized, transferred to plastic cages containing sawdust bedding (five mice/3 rats per cage), and quarantined for 1 week. Animals were provided food (Purina certified laboratory chow) and water ad libitum. Animal holding rooms were kept at 21–24 °C and 40–60% humidity with a 12-h light/dark cycle. The Michigan State University Institutional Animal Care & Use Committee approved all experiments involving the use of animals.

### 2.4. Isolation of rat and mouse B cells

Mouse or rat B cells were isolated from spleens of female C57BL/6 mice or Sprague-Dawley rats, and were made into single-cell suspensions by passage through a 40 μm cell strainer (BD Biosciences, San Jose, CA). Negative selection of rat or mouse B cells was conducted using MACS Naive Rat B cell, or Mouse B Cell Isolation Kits following the manufacturer's protocols (Miltenyi Biotec, Auburn, CA) and as described previously (Lu et al., 2009). In all cases, the purity of isolated B cells was ≥95%.

### 2.5. Preparation of the luciferase reporter construct

pGL3-basic firefly luciferase reporter vector containing the 5′-flanking region from −1523 to +20 of the human Cyp1B1 gene was kindly provided by Dr. Weiguo Han of the Albert Einstein College of Medicine. The 5′-flanking region of the human Cyp1B1 and luciferase were transferred into the pLEX-MCS lentiviral vector (Thermo Scientific, Waltham, MA). Nucleotide sequences were confirmed by DNA sequencing analyses.

### 2.6. Luciferase assays

For transient transfections, HEK293T cells were plated at $1.2 \times 10^6$ cells/well in 6-well plates and transfected with 6 μg of the Cyp1B1-luciferase constructs using lentiviral packing mix (Open Biosystems, Huntsville, AL) according to the manufacturer's instructions. After a 16 h incubation, HEK293T cells were co-cultured with $2 \times 10^6$ SKW cells/well for an additional 24 h. After 24-h of co-culture, SKW cells were separated from HEK293T cells and treated with vehicle (0.01% DMSO) or TCDD (30 nM) for 24 h. The cells were then washed with 1× phosphate-buffered saline and lysed with 1× reporter lysis buffer (Promega, Madison, WI). Samples were immediately frozen at −80 °C. To measure luciferase enzyme activity, samples were thawed, and 20 μl of sample lysate was mixed with 100 μl of luciferase assay reagent (Promega, Madison, WI) using an autoinjector. Luciferase activity was measured by KC-4 automated microplate reader (Bio-Tek, Winooski, VT) and represented as relative light units. Luciferase activity was normalized to the amount of protein determined by Bradford reaction (Protein Assay Kit, Pierce). Results are shown as fold induction determined by normalizing activation of different groups against VH control.

### 2.7. Preparation of stably expressing AhR cell lines

HEK293T cells were used for the transfection of the recombinant control and polymorphic human AhR fused to GFP as described above. After a 48 h co-culture incubation media was replaced, target cells were separated and cultured at 37 °C to approximately 80% confluence. Cells were then passaged and selected using RPMI media containing 0.5 μg/ml puromycin. Clones of the SKW cells were established using cloning by limiting dilution for 2 weeks in culture RPMI media containing 0.5 μg/ml puromycin. Individual SKW clones were screened for AhR mRNA expression and clones, expressing AhR mRNA at levels similar to that of primary human B cells, were selected for further characterization.