Published in final edited form as:

Toxicol Appl Pharmacol. 2017 February 01; 316: 95–106. doi:10.1016/j.taap.2016.11.009.

Comparative Analysis of TCDD-Induced AhR-Mediated Gene Expression in Human, Mouse and Rat Primary B Cells

Natalia Kovalova^{*,†}, Rance Nault^{‡,†}, Robert Crawford[†], Timothy R. Zacharewski^{‡,†}, and Norbert E. Kaminski^{*,†,1}

^{*}Department of Pharmacology and Toxicology, Michigan State University, Lansing, MI 48824, USA

[†]Institute for Integrative Toxicology, Michigan State University, Lansing, MI 48824, USA

[‡]Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, 48824, USA

Abstract

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent environmental pollutant that activates the aryl hydrocarbon receptor (AhR) resulting in altered gene expression. *In vivo*, *in vitro*, and *ex vivo* studies have demonstrated that B cells are directly impaired by TCDD, and are a sensitive target as evidenced by suppression of antibody responses. The window of sensitivity to TCDD-induced suppression of IgM secretion among mouse, rat and human B cells is similar. Specifically, TCDD must be present within the initial 12 h post B cell stimulation, indicating that TCDD disrupts early signaling network(s) necessary for B lymphocyte activation and differentiation. Therefore, we hypothesized that TCDD treatment across three different species (mouse, rat and human) triggers a conserved, B cell-specific mechanism that is involved in TCDD-induced immunosuppression. RNA sequencing (RNA-Seq) was used to identify B cell-specific orthologous genes that are differentially expressed in response to TCDD in primary mouse, rat and human B cells. Time course studies identified TCDD-elicited differential expression of 515 human, 2371 mouse and 712 rat orthologous genes over the 24-h period. 28 orthologs were differentially expressed in response to TCDD in all three species. Overrepresented pathways enriched in all three species included cytokine-cytokine receptor interaction, ECM-receptor interaction, focal adhesion, regulation of actin cytoskeleton and pathways in cancer. Differentially expressed genes functionally associated with cell-cell signaling in humans, immune response in mice, and oxidation reduction in rats. Overall, these results suggest that despite the conservation of the AhR and its signaling mechanism, TCDD elicits species-specific gene expression changes.

¹To whom correspondence should be addressed at Institute for Integrative Toxicology, Michigan State University, 1129 Farm Lane, Room 315, Food Safety and Toxicology Building, East Lansing, MI 48824. Fax: (517) 355-4603. kamins11@msu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

TCDD; AhR; B cell; IgM

INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the prototypical aryl hydrocarbon receptor (AhR) ligand and environmental toxicant, is formed by combustion processes and due to its lipophilic nature tends to bioaccumulate in the food chain (Tuppurainen *et al.*, 2003). A common consequence of TCDD exposure in many species is the suppression of humoral immunity, of which the immunoglobulin M (IgM) response is highly sensitive (Dooley and Holsapple, 1988; North *et al.*, 2010; Lu *et al.*, 2011). For robust IgM antibody responses, B cells undergo rounds of activation, proliferation and plasmacytic differentiation into antibody forming cells (AFC), which synthesize and secrete large quantities of pentameric IgM.

Pokeweed mitogen (PWM), a lectin purified from *Phytolacca Americana* is one of the most commonly used B cell polyclonal activators. PWM acts in a MyD88-dependent manner to drive B cell differentiation into IgM antibody secreting plasma cells in rodents and humans (Farnes *et al.*, 1964; Bekeredjian-Ding *et al.*, 2012). Significant changes in morphology, phenotype, and gene expression accompany B cell to plasma cell differentiation. Multiple critical transcriptional regulators control plasmacytic differentiation at the molecular level, some of which have been recently identified. Importantly, TCDD has been demonstrated to directly and indirectly deregulate mRNA and/or protein expression of AP-1, Bach-2, BCL-6, Pax5, and Blimp1, all of which are major regulators of the B cell differentiation program (Calame *et al.*, 2003; Igarashi *et al.*, 2007; De Abrew *et al.*, 2011; Phadnis-Moghe *et al.*, 2015; North *et al.*, 2010).

At the cellular level, TCDD directly suppresses the IgM response in human, mouse and rat B cells with maximal suppression reaching approximately 50% of the vehicle control response. Additionally, the window of sensitivity during which TCDD can suppress IgM secretion is similar between mouse, human and rat B cells, suggesting a common mechanism of action of IgM suppression. Specifically, TCDD must be added to the activated B cells within the initial 12 h post-stimulation to suppress IgM secretion (Dooley and Holsapple, 1988; Sulentic *et al.*, 1998; Kovalova *et al.*, 2016). The relatively narrow window of sensitivity suggests TCDD-mediated interference with a critical event shortly following B-cell activation.

Signaling through the aryl hydrocarbon receptor (AhR), a member of the Per-Arnt-Sim (PAS) family of transcription factors, is required in order for TCDD to suppress the primary IgM response, as this response is not observed in AhR null B cells (Sulentic *et al.*, 1998; Vorderstrasse *et al.*, 2001). In the absence of a ligand, the AhR is maintained in the cytosol bound by a number of chaperone proteins such as HSP90 and ARA9 (also known as XAP2 or AIP) (Petrulis and Perdew, 2002). Following TCDD binding, the AhR translocates to the nucleus where it forms heterodimers with AhR nuclear translocator (ARNT). The AhR-ARNT complex binds to dioxin response elements (DREs), modulating the transcription of a

number of target genes (Hankinson *et al.*, 1995; Denison *et al.*, 1998). However, the exact mechanism by which TCDD impairs the B cell IgM response and the level of its conservation between species remain largely unknown.

Interestingly, in the context of B cell function, TCDD has been shown to suppress the IgM response to a comparable level in mouse, human and rat primary B cells, suppress human and mouse B cell activation markers including CD80, CD86 and CD69, and suppress the activation of ERK, JNK, and Akt in mouse and human primary B cells (Lu *et al.*, 2010 and 2011; North *et al.*, 2010). Collectively, these data indicate that TCDD likely acts via a common rather than species-specific pathway to exert IgM suppression. The common effects of TCDD on human and mouse primary B cell activation and antibody secretion may represent important mechanisms of TCDD-elicited attenuation of B cell effector function among different species.

The objective of the present study was to characterize the effects of TCDD-dependent AhR activation on gene expression in primary mouse, rat and human B cells under the same conditions of activation (i.e., PWM) to identify potential molecular targets of TCDD. Overall, the global gene expression profiling at different time points identified multiple common and species-specific gene expression changes in response to TCDD. Interestingly, cytokine activity, cell adhesion and the inflammatory response were among the biological processes that were enriched in human, mouse and rat primary B cells. Additionally, regulation of actin cytoskeleton, focal adhesion, and ECM-receptor interaction KEGG pathways were enriched in all three species. Collectively, gene ontology and pathway enrichment analyses demonstrate that TCDD might act via multiple pathways to suppress IgM response.

MATERIALS AND METHODS

Chemicals and reagents

TCDD in dimethyl sulfoxide (DMSO) (purity 99.1%) was purchased from AccuStandard Inc (New Haven, CT). DMSO and pokeweed mitogen (PWM) (Lot. O L8777-5MG) were purchased from Sigma-Aldrich (St. Louis, MO).

Animals

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/three 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.

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%. Purified B cells were cultured at a density of 1×10^6 cells/ml in RPMI-1640 supplemented with 10% bovine calf serum, 100 U/ml penicillin/streptomycin, and 50 μ M 2-mercaptoethanol at 37°C, 5% CO₂/95% air, 98% humidity. Cell viability was assessed by trypan blue exclusion and found to exceed 90% for all experiments with primary B cells.

Human leukocyte packs

Human leukocyte packs collected from anonymous female donors were purchased from the Gulf Coast Regional Blood Center (Houston, TX). All donors were screened for human immunodeficiency virus and hepatitis at the blood center.

Isolation of the naïve human B cells

Human naïve (CD19⁺ CD27⁻) B cells were isolated from peripheral blood mononuclear cells (PBMCs) enriched from each leukocyte pack by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ). Negative selection of human B cells was conducted using MACS Naive human B-cell Isolation Kit 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 above 95%. Purified B cells were cultured at a density of 1×10^6 cells/ml in RPMI-1640 supplemented with 10% bovine calf serum, 100 U/ml penicillin/streptomycin, and 50 μ M 2-mercaptoethanol at 37°C, 5% CO₂/95% air, 98% humidity. Cell viability was assessed by trypan blue exclusion and found to exceed 90% for all experiments with primary B cells.

Whole transcriptome expression profiling via RNA-Seq

Total RNA was isolated from mouse, human and rat primary B cells activated with PWM (15 μ g/ml) and treated with vehicle (0.02% DMSO) or TCDD (30 nM) for 4, 8 and 24 h using RNeasy Kit (Qiagen, Valencia, CA) with gDNA eliminator columns. It is known that, depending on the activation stimulus, B cells in one species respond more strongly than in another (e.g., LPS). The rationale for the use of PWM to activate mouse, human and rat primary naïve B cells was based on the fact that PWM allows rapid activation of large numbers of B cells in all three species used in this study. Additionally, levels of the AhR and its affinity for TCDD differ approximately 10-fold between human and mouse (Ramadoss and Perdew, 2004), representing another potential confounder in these studies. Therefore, a high concentration of TCDD (30 nM) was selected to ensure strong activation of the AhR in all three species and subsequently strong induction or repression of AhR-regulated genes. RNA sample quality was assessed using Bioanalyzer RNA Nano chips (Agilent); all RNA samples had an RNA Integrity Number greater than or equal to eight.

RNA-Sequencing, alignment, and analysis

RNA-Sequencing was performed at the Michigan State University Research Technology Support Facility Genomics Core (RTSF, <https://rtsf.natsci.msu.edu/genomics>). In summary,

libraries from three human donors (N = 3) and a pool of 20 mice and 5 rats were prepared using the Illumina TrueSeq RNA Sample Preparation Kit (Illumina, San Diego, CA) according to manufacturer's instructions. Library sizes were confirmed using Caliper GX (Perkin Elmer, Waltham, MA), and quantified by qPCR using the Kapa Biosystems quantification kit (Wilmington, MA). Sequencing of libraries was performed at an average depth of 30M each on an Illumina HiSeq 2500.

The Michigan State High Performance Computer (MSU HPCC; <https://icer.msu.edu/hpcc>) was used for read processing and analysis. Reads, 1×50 bp with a seven-base index, were demultiplexed and quality was determined using FASTQC v0.11.2 (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adaptor sequences were removed using Cutadapt v1.4.1 (Martin, 2011) and low-complexity reads were cleaned using FASTX v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads were mapped to the mouse (GRCm38 release 81), rat (Rn5 release 76), and human (GRCh38 release 76) reference genomes using Bowtie 1.0.0 and TopHat v1.4.1 (Langmead et al., 2009) using default parameters and a minimum and maximum intron length of 10 and 15000, respectively. Alignments were converted to SAM format using SAMTools v0.1.19. (samtools.sourceforge.net/). Gene counts were determined using HTSeq v0.6.1 in intersection-nonempty mode (`-m intersection-nonempty`). RNA-Seq data is deposited in Gene Expression Omnibus (GEO; accession number GSE80953). Counts were transformed through variance stabilizing transformation (VST) using the DESeq package in R (www.r-project.org) according to the DESeq reference manual. Data was normalized using a semi-parametric approach (Eckel et al., 2004) in SAS v9.3 (SAS Institute Inc., Cary, NC). Posterior probabilities $P1(t)$ values were calculated for human datasets using an empirical Bayes method based on a per gene and dose basis using model-based t values as previously described (Eckel et al., 2004; Nault et al., 2015).

Network and functional ontology enrichment analysis

Functional enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, <http://david.abcc.ncifcrf.gov>) (Dennis et al., 2003) filtered for gene ontology biological processes (BP), molecular functions (MF), and cellular component (CC). Functional categories were considered enriched when the $-\log$ scale geometric mean p -value ≥ 0.05 (enrichment score ≥ 1.3).

Signaling network was constructed to connect 114 common orthologs deregulated by TCDD in human and mouse datasets. The network was constructed using GeneGo Metacore database (GeneGo, Inc., St Joseph, MI) with a maximum number of steps set to 2.

Identification of putative dioxin response elements

Identification and scoring of putative DREs (pDREs) core in mice was previously published (Nault et al., 2015). For rat and human pDREs, identification and scoring was performed as previously described (Dere et al., 2011; Nault et al., 2015). Briefly, bona fide DREs consisting of a 5'-GCGTG-3' core identified in Nault et al., 2015, and the upstream and downstream 7bp flanking sequence were used to generate a position weight matrix. Using the UCSC genome database and the UCSC tool findMotif, all DREs were extracted from the

rat (Rn5) and human (hg38) genomes. The UCSC tool twoBitToFa was then used to extract sequences and flanking 5' and 3' regions which were then used to calculate a matrix similarity score (MSS) as previously described (Dere et al., 2011) (Supplementary table 1).

Quantitative real-time PCR

Total RNA was isolated using the RNeasy Kit (Qiagen, Valencia, CA) and RNA concentrations were quantified using a Nanodrop ND-1000 spectrophotometer (Wilmington, DE). Double stranded cDNA was synthesized using 500 ng of total RNA using the Applied Biosystems high capacity cDNA reverse transcription kit (Foster City, CA). qRT-PCR was performed according to manufacturer's instructions using the Taqman Universal PCR Master Mix and Taqman gene expression assays for human CYP1A2 (Hs01070374_m1), CYP1B1 (Hs00164383_m1), CD27 (Hs00386811_m1), CD93 (Hs00362607_m1), LCK (Hs00178427_m1), SERPINB2 (Hs01010736_m1), FOSB (Hs00171851_m1), MTSSL1L (Hs00416413_m1), ITGB3 (Hs01001469_m1), RND2 (Hs00183269_m1), HIC1 (Hs00359611_s1), SHF (Hs00403125_m1), BEND7 (Hs00381740_m1). All qRT-PCR measurements were made on an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The change in gene expression was calculated using the $\Delta\Delta C_t$ method using 18S ribosomal RNA (4319413E) as an internal control (Livak *et al.*, 2001). For statistical analysis, unpaired two-tailed Student's t-tests were performed between treatments and their corresponding controls.

RESULTS

Gene expression changes in PWM-activated primary mouse, rat and human B cells following TCDD treatment

Human datasets were filtered using a $|\text{fold-change}| \geq 1.5$ and $P(t) \leq 0.05$ criteria while mouse and rat data sets were filtered using $|\text{fold-change}| \geq 1.5$ criterion. No $P(t)$ value was calculated for the mouse and rat data since the samples were pooled from 20 mice and 5 rats. Analysis of the time-course RNA-Seq results identified TCDD-elicited differential expression of 544 human, 2527 mouse, and 772 rat genes over the 24-h time period. In the mouse dataset approximately the same number of genes were up- or down-regulated in response to TCDD (Figure 1A). In the human dataset slightly more genes were up-regulated in response to TCDD at 8h and twice as many genes were up-regulated than down-regulated at 24h. By contrast, in the rat dataset significantly more genes were down-regulated than up-regulated at 8 and 24h. Specifically, up-regulation was observed for 1493 mouse, 308 rat, and 338 human genes; down regulation was observed for 1451 mouse, 523 rat and 217 human genes (Figure 1A). Comparison of the top ten differentially expressed genes among the three species, revealed one common up-regulated gene - *CYP1A1* but no common down-regulated genes (Table 1). Additionally, immunoregulatory genes *IL22* and *Pde4c* were up-regulated in mouse and rat, *AHRR* and *CYP1B1* orthologs in mouse and human, and *PACSLN3* in the human and rat datasets. Conversely, only human and mouse datasets shared a down-regulated ortholog, *TMEM40* (Table 1). All data is deposited on GEO (GSE80953).

Mapped orthologs were analyzed using HID (HomoloGene ID; HID build 67) in order to compare TCDD-induced differential expression of equivalent genes across human, rat and

mouse primary activated B cells. The HomoloGene database contains 18,981 human, 21,766 mouse and 19,229 rat unique HIDs, of which 15,816, 16,461 and 14,145 HIDs were expressed in primary B cells. In the time course study, 30 nM TCDD elicited the differential expression of 515 human, 2371 mouse, and 712 rat orthologs. 28 orthologs were identified as differentially expressed in response to TCDD in all 3 species. The majority of orthologs exhibited species-specific expression. Specifically, 73% (379/515) of human, 81% (1935/2371) of mouse, and 50% (350/712) of rat orthologs exhibited species-specific differential expression (Figure 1B). Ten of the 28 orthologs differentially expressed in all 3 species, exhibited a comparable expression pattern (Table 2). This included the induction of prototypical AhR battery genes such as *CYP1A1*, *TIPARP*, *AHRR*, as well as induction of *CD27*, a memory B cell marker, the actin binding gene, *MTSS1L*, the *MYO6* gene that plays a role in intracellular vesicle and organelle transport, and the potassium-dependent sodium/calcium exchanger, *SLC24A3*. Calcium-binding protein, *CABYR*, *CHRM4* involved in regulation of the actin skeleton, and a G protein-coupled receptor, *P2RY13*, genes were down-regulated in all 3 species. However, most of the orthologs (64%) differentially regulated by TCDD in all three species, exhibited divergent expression. For example, the transcriptional repressor, *Hic1*, *Rnd2* involved in disassembly of actin and cell surface signaling, and *Itgb3* were induced in mouse and human but suppressed in rat primary B cells. The actin binding protein, *Fscn2*, and *Pacsin*, involved in endocytosis, were induced in human and rat but suppressed in the mouse. Additionally, transcriptional repressor, *Hey2*, and *Map6*, involved in microtubule stabilization, were induced in rodents but suppressed in human.

Pairwise comparisons identified 86 common differentially regulated human-mouse orthologs and 12 common human-rat orthologs. Interestingly, the kinetics of gene expression change in response to TCDD was different between mouse and human resulting in 45 common up-regulated and 21 down-regulated orthologs at least at one time point. Among the 12 common human-rat orthologs 6 were down-regulated, 4 up-regulated and 2 displayed divergent gene expression changes (Figure 2A and B). No significant correlation between any two species was identified by pairwise comparison (data not shown).

The functional relevance of 86 common mouse and human significantly changed genes was examined using Database for Annotation, Visualization and Integrated Discovery (DAVID). Results for the top 25 gene ontology (GO) biological functions (which includes 3 classes: molecular function, biological processes and cellular components) are displayed in table 3. The three top GO terms include extracellular matrix, integrin-mediated signaling pathway and calcium ion binding.

Functional annotation and pathway enrichment of genes differentially expressed in response to TCDD in activated mouse, human and rat primary B cells

In order to determine functional relevance of DEGs the 2527 mouse, 772 rat, and 544 human genes were analyzed for overrepresentation within specific functional categories and canonical pathways using the DAVID analysis tool. The top enriched pathways and biological processes specific to mouse, rat and human primary B cells are presented in figures 3 and 4. The number of overrepresented GO terms (DAVID) was comparable (311

mouse, 294 rat and 184 human enriched functional categories) in the time course data sets. The enriched categories were compared between species identifying 41 common enriched categories, including cell adhesion, extracellular region and the inflammatory response. Species-specific enriched functions included chemotaxis and positive regulation of MAP kinase activity in mouse primary B cells, mitochondrion and oxidation reduction in rat primary B cells, and integrin binding and cell-cell signaling in human primary B cells (complete list of overrepresented functions is presented in supplementary table 2). Importantly, the number of genes within functional categories varied among species and direction of gene changes was frequently discordant between species despite the consistent phenotypic consequence of TCDD exposure – suppression of the IgM response (Figure 5). For example, genes involved in cell adhesion, *CCR1* and *DST*, were induced in human but suppressed in mouse and rat. Conversely, genes involved in cell differentiation *BOC*, *RUNX2*, *COL18A1*, and *MYO6* were up-regulated in all three species (Figure 3).

TCDD-induced differential gene expression mapped to 34 mouse, 25 rat and 27 human KEGG canonical pathways, among which 5 were common between mouse, human and rat. Top ranking mouse, rat and human pathways are presented in figure 4. Pathways significantly enriched in all three species included ECM-receptor interaction, focal adhesion and regulation of actin cytoskeleton. Mouse-specific differential gene expression was associated with acute myeloid leukemia and toll-like receptor signaling pathway (supplementary table 3). In the rat primary B cells differentially expressed genes mapped to oxidative phosphorylation and mTOR signaling pathway (supplementary table 3). Majority of the genes within enriched rat-specific pathways were down-regulated including mitochondrial ATP synthase subunit *Atp5d*, subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) *Ndubf1*, *Ndubf2* and *Ndubf3* and nuclear-coded polypeptide chains of cytochrome c oxidase *Cox8a*, *Cox6a1* and *Cox5a*. In human primary B cells the specific significantly enriched canonical pathways included ribosome and metabolism of xenobiotics by cytochrome pathway (supplementary table 3). Importantly, genes within common enriched pathways frequently displayed divergent pattern of deregulation.

Temporal gene expression changes in the PWM-activated primary mouse, human and rat B cells following TCDD exposure

Time-course analysis of the data revealed that the standard AhR battery genes (i.e., *CYP1A1*, *CYP1B1*, *NQO1*, *TIPARP*) showed an unusual and divergent gene expression pattern (Figure 6). In many well-characterized cell models, for example HepG2, activation of metabolizing enzymes *CYP1A1* and *CYP1B1* is robust and persistent. However, in primary human and mouse B lymphocytes *CYP1B1* mRNA expression peaks as soon as 4h post-TCDD treatment and gradually decreases over time. Human *CYP1A1* showed gradual time-dependent increase while in mouse and rat primary B cells *Cyp1a1* expression peaked at 4h following TCDD treatment.

Enrichment analysis was performed on the differentially expressed gene sets at each time point to access TCDD-mediated time-dependent changes in biological and molecular functions (supplementary table 4). In human, biological processes related to cell adhesion

and cell-cell signaling showed high enrichment at 4h followed by a decline at 24h. Molecular function associated with pattern binding and KEGG pathway associated with focal adhesion remained enriched during the time-course. In the mouse, biological processes involved in locomotory behavior were highly enriched at 4 and 8h post TCDD treatment while inflammatory response associated biological processes became enriched at later time points; KEGG pathway associated with cytokine-cytokine receptor interaction was enriched at all time points. In the rat, biological functions associated with cell adhesion and response to extracellular stimulus demonstrated peak enrichment at 4h; KEGG pathway associated with adhesion was enriched at all time points while Jak-STAT signaling pathway was significantly enriched only at the earliest time-point.

Identification of putative DREs in responsive genes

Genomic sequences for genes presented in table 2 and figure 2 were examined for presence of putative DREs, which were identified by computational scanning. Of the 86 common mouse-human orthologs 85 mouse and 86 human orthologs contained a pDRE with an MSS 0.8 (high scoring pDRE) while 29 mouse and 34 human orthologs contained pDREs with an MSS 0.9 (very high scoring pDRE) within its genomic region or 10 kb upstream of the transcription start site. Between 12 human-rat orthologs 11 rat and 12 human contained “high” scoring pDREs but only 3 rat and 6 human orthologs contained “very high” scoring pDREs. 27 out of 28 human and all mouse and rat orthologs deregulated in the three species contained “high” scoring pDREs; “very high” pDREs were mapped to 13 mouse, 10 rat and 10 human orthologs. *CD27*, *HIC1* and *MTSS1L* all possessed DREs within their promoter region and displayed an early induction response to TCDD treatment, suggesting that they are primary AhR-responsive genes.

Gene network analysis

To discover potential mechanisms of TCDD-mediated suppression of B cell function, a subgroup of 114 common mouse and human orthologs was evaluated with MetaCore. Thresholds of 1.5 (fold change) and $P(1) 0.8$ were applied and dataset was analyzed with Compare Experiments followed by network building option “Analyze Network (Transcription Factor)”, which incorporates canonical pathways in its algorithm. The resulting most significant network is presented in figure 7. This network shows interaction of two key transcriptional regulators CREB1 that, if activated in B cells, promotes proliferation and survival, and AP-1 a transcription factor that had been shown to suppress B cell to plasma cell differentiation in primary mouse B cells by directly binding to and inhibiting PRDM1 (Grotsc *et al.*, 2014).

Validation of differentially expressed genes at the RNA level

Differential expression of 13 genes in human primary B cells was validated by quantitative real-time PCR (Figure 8). In order to strengthen the comparison between RNA-Seq and qRT-PCR, PCR was carried out using the RNA samples that were used for RNA-Seq experiments plus two additional human donors. Two genes from AhR battery of genes (*TIPARP* and *CYP1A1*) and 11 genes deregulated in more than one species were chosen for confirmation. Differential expression was confirmed for all genes and the fold change calculated with the qRT-PCR data showed mostly a similar magnitude of change compared to the fold change

calculated with the RNA-Seq data. qRT-PCR fold change was significantly greater than RNA-Seq fold change only for CYP1A1 gene; however, the direction of gene expression change was the same.

DISCUSSION

The B lymphocyte is a sensitive cellular target of TCDD-induced AhR-mediated toxicity, specifically, suppression of the IgM response (Sulentic and Kaminski, 2011). Studies in mouse, rat and human B lymphocytes show similar levels both in terms of the magnitude of TCDD-induced IgM suppression as well as similarity in time-of-addition kinetics (Kovalova et al., 2016). Concordance across all three animal species suggested a common mode of action. TCDD-mediated, AhR-dependent deregulation of gene expression is assumed to be a primary mode of action for TCDD-induced toxicity (Birnbaum and Tuomisto, 2000). The underlying hypothesis of this study was that TCDD deregulates a core set of orthologous genes in mouse, rat and human primary B cells at early time points after B cell activation. To evaluate this hypothesis, comprehensive genome-wide time course gene expression was measured in primary mouse, human and rat B cells activated with PWM and treated with a single dose of TCDD. Many previous studies examining TCDD-induced suppression of the IgM response have focused on individual genes such as Pax5, Blimp1, BCL-6 and Bach2 or used transformed cell lines that may not completely recapitulate primary B cell responses (North et al., 2010; Lu et al., 2010; DeAbrew et al., 2011; Phadnis-Moghe et al., 2015). Therefore, use of primary B cells provided a unique opportunity to investigate and compare gene expression changes in response to TCDD treatment in different species and to identify additional B cell-specific targets of TCDD.

Comparative genome-wide gene expression analysis identified a limited number of candidate orthologs deregulated by TCDD in all three species and a large number of divergent and species-specific responses elicited by TCDD across human, mouse and rat primary B cells. In agreement with previous comparative analyses performed in hepatocytes, most of the TCDD-mediated gene expression changes showed species-specific deregulation (Forgacs *et al.*, 2013; Dere *et al.*, 2011; Nault *et al.*, 2013). For example, only 16 orthologs were differentially expressed in human, mouse and rat primary hepatocytes following treatment with 10 nM TCDD for 48h (Forgacs *et al.*, 2013). In the current study, TCDD elicited limited overlap between human, mouse and rat primary B cell differential gene expression with only 28 orthologs in common between all three species. This included the induction of “AhR battery” genes such as *Cyp1a1*, *Tiparp* and *Ahr*. Notably, the fold induction of *Cyp1A1* and *Cyp1B1* mRNA levels in primary B cells was low compared to hepatocytes and is likely due, at least in part, to the fact that the primary role of B cells is defense against pathogens and not drug metabolism. This statement is supported by the fact that B cells in general express low levels of phase I and phase II drug metabolizing enzymes. In addition, AhR mRNA levels are low in primary B cells, but rapidly and transiently (i.e., over the first 24 hours) increased post activation followed by a rapid decline to background levels by 48 hours post-activation (Allan and Sherr, 2005; Crawford et al., 1997). By contrast, in other cell types such as hepatocytes, AhR levels are maintained at significantly higher levels than the B cell.

Promoter analyses showed that all common orthologs have AhR binding sites in their promoter region. Interestingly, *CD27*, *HIC1* and *MTSS1L* genes all have high scoring pDREs and are activated in response to TCDD in all three species suggesting these genes are direct targets of AhR. *CD27* encodes a member of the TNF-receptor superfamily that activates NF- κ B and MAPK8/JNK signaling and plays a role in B cell activation and immunoglobulin synthesis (Akiba *et al.*, 1998; Agematsu *et al.*, 1995; Lens *et al.*, 1995). *HIC1* encodes a sequence-specific transcriptional repressor that directly targets ATOH1 (a pro-neuronal transcription factor), CXCR7 (a receptor for the chemokine CXCL12), and ephrin-A1 (a cell surface ligand for Eph receptors) and regulates p53- and E2F1-dependent cell survival and growth control (Van Rechem *et al.*, 2009; Zhang *et al.*, 2010). *HIC1* transcription factor recruits multiple co-repressors, including CtBP (a co-repressor for BCL6 autoregulation), possibly targeting genes involved in B cell proliferation and survival, or preventing B cell differentiation by targeting PRDM1 via MTA3/NuRD co-regulators (Ci *et al.*, 2008; Van Rechem *et al.*, 2010). *MTSS1L* (ABBA) is an actin dynamics regulator that controls actin and plasma membrane dynamics in radial glial cells, plays a critical role in Rac1-mediated cell spreading in the NIH3T3 cells, and is essential for Rac1 activation (Saarikangas *et al.*, 2008; Zeng *et al.*, 2013). Rac1, a small signaling GTPase, is ubiquitously expressed and regulates multiple processes including cell adhesion, transcription, proliferation, and differentiation (Rose *et al.*, 2007; Chae *et al.*, 2008; Bosco *et al.*, 2009). The exact role of the TCDD-induced deregulation of *CD27*, *HIC1* and *MTSS1L* genes in suppression of the IgM response should be further evaluated.

Interestingly, most of the genes that were differentially regulated by TCDD were so in a species-specific manner. For example, in the mouse dataset, the most up-regulated transcript, not including drug metabolizing enzyme genes, was *Serpinb2*, a plasminogen activator inhibitor type 2 (PAI-2). *Serpinb2* is up-regulated under multiple inflammatory conditions and might be involved in regulation of the adaptive immune response since it had been demonstrated that *Serpinb2*^{-/-} mice generate increased Th1 responses (Schroder *et al.*, 2011). The most down-regulated transcript was methionine sulfoxide reductase B3 (*MsrB3*), an endoplasmic reticulum oxidoreductase that regulates cell growth through the p53–p21 and p27 pathways (Lee *et al.*, 2014). A number of genes involved in regulation of signal transduction (*Il22*, *Mcf2l*, *Psd2* and *Thbs1*) were among the top 10 up-regulated, and genes associated with the immune response (*Cd70*, *FasI*, H2-B1, *Sh2d1a*) were among the most down-regulated transcripts in the mouse dataset. In the rat dataset, genes associated with Jak-STAT signaling pathway (*Ifnb1*, *Il22*, *Pik3r3*, *Spred3*) were among the top up-regulated, and genes associated with cell differentiation (*Ctbp2*, *Cebpb*, *Tbx6*, *Fscn3*, *Rps19*, *Spr*, *Socs1*) were among the most down-regulated. The most strongly expressed genes in human primary B cells were associated with signal transduction (*ARL4C*, *GPR68*, *ASB2*, *CLNK*, *GRIN2D*); the transmembrane transport genes (*SLC16A14*, *SLC13A3*, *SLC28A2*, *SLC5A9*, *SPNS3*) were among the most down-regulated. The most up-regulated gene, *CD93* (encodes for the CD93 molecule), is important for the maintenance of plasma cells in the bone marrow; CD93-deficient mice fail to maintain bone-marrow plasma-cell numbers and antibody secretion (Chevrier *et al.*, 2009). The most down-regulated gene, *TMEM40*, encodes for uncharacterized protein TMEM40 that has been associated with arthritis in mice, suggesting a potential role in inflammation (Macauley *et al.*, 2007; Yu *et al.*, 2009).

Gene ontology and pathway enrichment analyses were performed to compare, group and functionally interpret conserved and species-specific gene expression changes elicited by TCDD in primary mouse, human and rat B cells. Functional analysis identified cytokine activity, cell adhesion and inflammatory responses among the biological processes that were enriched in human, mouse and rat primary B cells. Additionally, multiple KEGG pathways including focal adhesion, ECM-receptor interaction, and regulation of actin cytoskeleton were enriched in all three species. It is tempting to speculate that TCDD-induced deregulation of abovementioned processes results in the suppression of the IgM response especially considering that proper cell adhesion and regulation of actin cytoskeleton are paramount for receptor signaling and immunological synapse formation (Mattila et al., 2016; Wickramarachchi et al., 2010). Interestingly, even though comparable functions and pathways were affected between the species, different orthologs within common pathways and biological processes were differentially regulated indicating that TCDD may use species-specific mechanisms to suppress the IgM response. For example, among the KEGG pathways enriched in rat were oxidative phosphorylation and the Jak-STAT signaling pathway. The Jak-STAT pathway plays a role in the initiation of B cell differentiation through activation of *Prdm1* and down-regulation of *Bcl6* (Desrivieres et al., 2006; Diehl et al., 2008; Klein et al., 2003; Riley et al., 2005). AhR affects Jak-STAT signaling at the early time points and may play a role in suppression of B-cell activation and differentiation in the rat primary B cells. In the human primary B cells, TGF- β and calcium signaling KEGG pathways were enriched. TGF- β is a well-known potent immunosuppressor. It has previously been demonstrated that TGF- β inhibits proliferation and survival of B cells, blocks progression through the cell cycle and inhibits secretion of IgM (Lebman and Edmiston, 1999; Kehrl et al., 1991). Additionally, it has been previously demonstrated that lowering intracellular calcium levels inhibits IgM secretion (Tartakoff et al., 1977). In the mouse primary B cells, pathways associated with chemokine signaling and ABC transporters were enriched. The pattern of expression and function of ABC transporters on B cells is not well understood, but recent reports have demonstrated that ABC transporters play important roles in the differentiation, and maturation of multiple types of immune cells (Van de Ven et al., 2009). Collectively, gene ontology and pathway enrichment analyses demonstrate that TCDD might act via different species-specific pathways to suppress IgM response.

Interestingly, using MetaCore analysis, a cAMP-dependent transcription factor CREB1 and an activator protein 1 (AP1) were identified as top upstream transcriptional regulators. CREB1 involvement in the cAMP/PKA signaling and in the AhR signaling pathways had been previously demonstrated in multiple cell types, including in mouse B cells (Feng et al., 2016; Landers and Bunce, 1991). Similarly, it has been previously demonstrated that in mouse primary B cells, a member of AP1 transcription factors *Fra1* blocks plasma cell differentiation and IgM production by directly repressing *Blimp1* expression (Grotsch et al., 2014). Together, these data indicate that TCDD, likely, acts via multiple and species-specific pathways to produce IgM secretion suppression in mouse, human and rat B cells.

Importantly, study design limitations such as quality of the genome builds, accuracy and number of annotated genes, media composition, use of PWM as an activating stimulus may have limited the overall interspecies comparison and biased data interpretation. Specifically,

human and mouse genomes are more complete and have a higher number of annotated genes as compared with the rat. Furthermore, the exact mechanism of PWM-induced B cell proliferation and IgM secretion is not well understood and exact early signaling events may vary between species (Bekeredjian-Ding et al., 2012). Additionally, this study included B cells from female human donors of undisclosed age; mouse and rat primary B cells were isolated from mature female rodents. Despite these biases, the hallmark measure of AhR activation, induction of metabolizing enzymes *Cyp1a1* and *Cyp1b1* was prompt and robust among species, indicating that the study design limitations likely did not have a detrimental effect on data interpretation.

In this study a comprehensive analysis of TCDD-induced transcriptomic changes in mouse, human and rat primary B cells was performed to compare and characterize common and species-specific gene expression and identify B cell molecular targets of TCDD. Importantly, this is the first study to investigate genome-wide responses in human, mouse and rat primary B cells treated with TCDD. Collectively, time-course comparison of mouse, human and rat genomic responses presented in this study suggest that despite the evolutionary conservation of the AhR and its signaling pathway (Hahn, 2002), there are significant differences in TCDD-regulated molecular mechanisms between species that disrupt early signaling during B cell activation and differentiation and ultimately lead to the suppression of the IgM response. Further studies will be needed to confirm the specific roles of deregulated genes and molecular mechanisms responsible for impaired B cell activation and plasmacytoid differentiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING INFORMATION:

This work was supported by the National Institute of Environmental Health Sciences Superfund Research Program (NIEHS SRP P42ES004911) and National Institute of Environmental Health Sciences grant (2R01 ES002520-25A1).

RN is supported by the MSU Barnett Rosenberg Endowed Assistantship and Integrative Training in the Pharmacological Sciences grant (NIH 5T32GM092715).

ABBREVIATIONS

TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
AhR	aryl hydrocarbon receptor
RNA-Seq	RNA sequencing
IgM	immunoglobulin M
AFC	antibody forming cells
PWM	Pokeweed mitogen

PAS	Per-Arnt-Sim
PBMCs	peripheral blood mononuclear cells
ARNT	AhR nuclear translocator
DREs	dioxin response elements
DMSO	dimethyl sulfoxide
GEO	Gene Expression Omnibus
VST	variance stabilizing transformation
DAVID	Database for Annotation, Visualization and Integrated Discovery
GO	gene ontology

References

- Agematsu K, Kobata T, Yang FC, Nakazawa T, Fukushima K, Kitahara M, Mori T, Sugita K, Morimoto C, Komiyama A. CD27/CD70 interaction directly drives B cell IgG and IgM synthesis. *Eur J Immunol.* 1995; 25:2825–2829. [PubMed: 7589078]
- Akiba H, Nakano H, Nishinaka S, Shindo M, Kobata T, Atsuta M, Morimoto C, Ware CF, Malinin NL, Wallach D, Yagita H, Okumura K. CD27, a member of the tumor necrosis factor receptor superfamily, activates NF-kappaB and stress-activated protein kinase/c-Jun N-terminal kinase via TRAF2, TRAF5, and NF-kappaB-inducing kinase. *J Biol Chem.* 1998; 273:13353–13358. [PubMed: 9582383]
- Allan LL, Sherr DH. Constitutive activation and environmental chemical induction of the aryl hydrocarbon receptor/transcription factor in activated human B lymphocytes. *Mol Pharmacol.* 2005; 67:1740–1750. [PubMed: 15681594]
- Bekeredjian-Ding I, Foermer S, Kirschning CJ, Parcina M, Heeg K. Poke weed mitogen requires Toll-like receptor ligands for proliferative activity in human and murine B lymphocytes. *PLoS One.* 2012; 7:e29806. [PubMed: 22238657]
- Birnbaum LS, Tuomisto J. Non-carcinogenic effects of TCDD in animals. *Food Addit Contam.* 2000; 17:275–288. [PubMed: 10912242]
- Bosco EE, Mulloy JC, Zheng Y. Rac1 GTPase: a “Rac” of all trades. *Cell Mol Life Sci.* 2009; 66:370–374. [PubMed: 19151919]
- Calame KL, Lin KI, Tunyaplin C. Regulatory mechanisms that determine the development and function of plasma cells. *Annu Rev Immunol.* 2003; 21:205–230. [PubMed: 12524387]
- Chae HD, Lee KE, Williams DA, Gu Y. Cross-talk between RhoH and Rac1 in regulation of actin cytoskeleton and chemotaxis of hematopoietic progenitor cells. *Blood.* 2008; 111:2597–2605. [PubMed: 18089848]
- Chevrier S, Genton C, Kallies A, Karnowski A, Otten LA, Malissen B, Malissen M, Botto M, Corcoran LM, Nutt SL, Acha-Orbea H. CD93 is required for maintenance of antibody secretion and persistence of plasma cells in the bone marrow niche. *Proc Natl Acad Sci U S A.* 2009; 106:3895–3900. [PubMed: 19228948]
- Ci W, Polo JM, Melnick A. B-cell lymphoma 6 and the molecular pathogenesis of diffuse large B-cell lymphoma. *Curr Opin Hematol.* 2008; 15:381–390. [PubMed: 18536578]
- Crawford RB, Holsapple MP, Kaminski NE. Leukocyte activation induces aryl hydrocarbon receptor up-regulation, DNA binding, and increased Cyp1a1 expression in the absence of exogenous ligand. *Mol Pharmacol.* 1997; 52:921–927. [PubMed: 9415701]
- De Abrew KN, Phadnis AS, Crawford RB, Kaminski NE, Thomas RS. Regulation of Bach2 by the aryl hydrocarbon receptor as a mechanism for suppression of B-cell differentiation by 2,3,7,8-

tetrachlorodibenzo-p-dioxin. *Toxicology and Applied Pharmacology*. 2011; 252:150–158. [PubMed: 21296099]

Denison MS, Heath-Pagliuso S. The Ah receptor: a regulator of the biochemical and toxicological actions of structurally diverse chemicals. *Bull Environ Contam Toxicol*. 1998; 61:557–568. [PubMed: 9841714]

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol*. 2003; 4:P3. [PubMed: 12734009]

Dere E, Lee AW, Burgoon LD, Zacharewski TR. Differences in TCDD-elicited gene expression profiles in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cells. *BMC Genomics*. 2011; 12:193. [PubMed: 21496263]

Desrivieres S, Kunz C, Barash I, Vafaizadeh V, Borghouts C, Groner B. The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. *J Mammary Gland Biol Neoplasia*. 2006; 11:75–87. [PubMed: 16947086]

Diehl SA, Schmidlin H, Nagasawa M, van Haren SD, Kwakkenbos MJ, Yasuda E, Beaumont T, Scheeren FA, Spits H. STAT3-mediated up-regulation of BLIMP1 is coordinated with BCL6 down-regulation to control human plasma cell differentiation. *J Immunol*. 2008; 180:4805–4815. [PubMed: 18354204]

Dooley RK, Holsapple MP. Elucidation of cellular targets responsible for tetrachlorodibenzo-p-dioxin (TCDD)-induced suppression of antibody responses: I. The role of the B lymphocyte. *Immunopharmacology*. 1988; 16:167–180. [PubMed: 3267010]

Eckel JE, Gennings C, Chinchilli VM, Burgoon LD, Zacharewski TR. Empirical bayes gene screening tool for time-course or dose-response microarray data. *J Biopharm Stat*. 2004; 14:647–670. [PubMed: 15468757]

Farnes P, Barker BE, Brownhill LE, Fanger H. Mitogenic Activity in *Phytolacca Americana* (Pokeweed). *Lancet*. 1964; 2:1100–1101. [PubMed: 14207901]

Feng Y, Tian J, Krylova I, Xu T, Xie HQ, Guo TL, Zhao B. Chronic TCDD exposure results in the dysregulation of gene expression in splenic B-lymphocytes and in the impairments in T-cell and B-cell differentiation in mouse model. *J Environ Sci (China)*. 2016; 39:218–227. [PubMed: 26899660]

Forgacs AL, Dere E, Angrish MM, Zacharewski TR. Comparative analysis of temporal and dose-dependent TCDD-elicited gene expression in human, mouse, and rat primary hepatocytes. *Toxicol Sci*. 2013; 133:54–66. [PubMed: 23418086]

Grotsch B, Brachs S, Lang C, Luther J, Derer A, Schlotzer-Schrehardt U, Bozec A, Fillatreau S, Berberich I, Hobeika E, Reth M, Wagner EF, Schett G, Mielenz D, David JP. The AP-1 transcription factor Fra1 inhibits follicular B cell differentiation into plasma cells. *J Exp Med*. 2014; 211:2199–2212. [PubMed: 25288397]

Hankinson O. The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol*. 1995; 35:307–340. [PubMed: 7598497]

Igarashi K, Ochiai K, Muto A. Architecture and dynamics of the transcription factor network that regulates B-to-plasma cell differentiation. *J Biochem*. 2007; 141:783–789. [PubMed: 17569706]

Kehrl JH, Thevenin C, Rieckmann P, Fauci AS. Transforming growth factor-beta suppresses human B lymphocyte Ig production by inhibiting synthesis and the switch from the membrane form to the secreted form of Ig mRNA. *J Immunol*. 1991; 146:4016–4023. [PubMed: 1903417]

Klein B, Tarte K, Jourdan M, Mathouk K, Moreaux J, Jourdan E, Legouffe E, De Vos J, Rossi JF. Survival and proliferation factors of normal and malignant plasma cells. *Int J Hematol*. 2003; 78:106–113. [PubMed: 12953803]

Kovalova N, Manzan M, Crawford R, Kaminski N. Role of aryl hydrocarbon receptor polymorphisms on TCDD-mediated CYP1B1 induction and IgM suppression by human B cells. *Toxicol Appl Pharmacol*. 2016; 309:15–23. [PubMed: 27535091]

Landers JP, Bunce NJ. The Ah receptor and the mechanism of dioxin toxicity. *Biochem J*. 1991; 276(Pt 2):273–287. [PubMed: 1646595]

Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol*. 2009; 10:R25. [PubMed: 19261174]

- Lebman DA, Edmiston JS. The role of TGF-beta in growth, differentiation, and maturation of B lymphocytes. *Microbes Infect.* 1999; 1:1297–1304. [PubMed: 10611758]
- Lee E, Kwak GH, Kamble K, Kim HY. Methionine sulfoxide reductase B3 deficiency inhibits cell growth through the activation of p53-p21 and p27 pathways. *Arch Biochem Biophys.* 2014; 547:1–5. [PubMed: 24583268]
- Lens SM, de Jong R, Hintzen RQ, Koopman G, van Lier RA, van Oers RH. CD27-CD70 interaction: unravelling its implication in normal and neoplastic B-cell growth. *Leuk Lymphoma.* 1995; 18:51–59. [PubMed: 8580829]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001; 25:402–408. [PubMed: 11846609]
- Lu H, Crawford RB, Suarez-Martinez JE, Kaplan BL, Kaminski NE. Induction of the aryl hydrocarbon receptor-responsive genes and modulation of the immunoglobulin M response by 2,3,7,8-tetrachlorodibenzo-p-dioxin in primary human B cells. *Toxicol Sci.* 2010; 118:86–97. [PubMed: 20702590]
- Lu H, Crawford RB, Kaplan BL, Kaminski NE. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated disruption of the CD40 ligand-induced activation of primary human B cells. *Toxicol Appl Pharmacol.* 2011; 255:251–260. [PubMed: 21807014]
- Macaulay IC, Tijssen MR, Thijssen-Timmer DC, Gusnanto A, Steward M, Burns P, Langford CF, Ellis PD, Dudbridge F, Zwaginga JJ, Watkins NA, van der Schoot CE, Ouwehand WH. Comparative gene expression profiling of in vitro differentiated megakaryocytes and erythroblasts identifies novel activatory and inhibitory platelet membrane proteins. *Blood.* 2007; 109:3260–3269. [PubMed: 17192395]
- Martin JA, Wang Z. Next-generation transcriptome assembly. *Nat Rev Genet.* 2011; 12:671–682. [PubMed: 21897427]
- Mattila PK, Batista FD, Treanor B. Dynamics of the actin cytoskeleton mediates receptor cross talk: An emerging concept in tuning receptor signaling. *J Cell Biol.* 2016; 212(3):267–280. [PubMed: 26833785]
- Nault R, Fader KA, Zacharewski T. RNA-Seq versus oligonucleotide array assessment of dose-dependent TCDD-elicited hepatic gene expression in mice. *BMC Genomics.* 2015; 16:373. [PubMed: 25958198]
- Nault R, Forgacs AL, Dere E, Zacharewski TR. Comparisons of differential gene expression elicited by TCDD, PCB126, betaNF, or ICZ in mouse hepatoma Hepa1c1c7 cells and C57BL/6 mouse liver. *Toxicol Lett.* 2013; 223:52–59. [PubMed: 23994337]
- North CM, Crawford RB, Lu H, Kaminski NE. 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated suppression of toll-like receptor stimulated B-lymphocyte activation and initiation of plasmacytic differentiation. *Toxicol Sci.* 2010; 116:99–112. [PubMed: 20348231]
- Petrulis JR, Perdew GH. The role of chaperone proteins in the aryl hydrocarbon receptor core complex. *Chem Biol Interact.* 2002; 141:25–40. [PubMed: 12213383]
- Phadnis-Moghe AS, Crawford RB, Kaminski NE. Suppression of human B cell activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin involves altered regulation of B cell lymphoma-6. *Toxicol Sci.* 2015; 144:39–50. [PubMed: 25543051]
- Ramados P, Perdew GH. Use of 2-azido-3-[125I]iodo-7,8-dibromodibenzo-p-dioxin as a probe to determine the relative ligand affinity of human versus mouse aryl hydrocarbon receptor in cultured cells. *Mol Pharmacol.* 2004; 66:129–136. [PubMed: 15213304]
- Riley RL, Van der Put E, King AM, Frasca D, Blomberg BB. Deficient B lymphopoiesis in murine senescence: potential roles for dysregulation of E2A, Pax-5, and STAT5. *Semin Immunol.* 2005; 17:330–336. [PubMed: 15967678]
- Rose DM, Alon R, Ginsberg MH. Integrin modulation and signaling in leukocyte adhesion and migration. *Immunol Rev.* 2007; 218:126–134. [PubMed: 17624949]
- Saarikangas J, Hakanen J, Mattila PK, Grumet M, Salminen M, Lappalainen P. ABBA regulates plasma-membrane and actin dynamics to promote radial glia extension. *J Cell Sci.* 2008; 121:1444–1454. [PubMed: 18413296]
- Schroder WA, Major L, Suhrbier A. The role of SerpinB2 in immunity. *Crit Rev Immunol.* 2011; 31:15–30. [PubMed: 21395508]

- Sulentic CE, Holsapple MP, Kaminski NE. Aryl hydrocarbon receptor-dependent suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin of IgM secretion in activated B cells. *Mol Pharmacol.* 1998; 53:623–629. [PubMed: 9547351]
- Sulentic CE, Kaminski NE. The long winding road toward understanding the molecular mechanisms for B-cell suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Sci.* 2011; 120(Suppl 1):S171–191. [PubMed: 20952503]
- Tartakoff AM, Vassalli P. Plasma cell immunoglobulin secretion: arrest is accompanied by alterations of the golgi complex. *J Exp Med.* 1977; 146:1332–1345. [PubMed: 925606]
- Tuppurainen K, Asikainen A, Ruokojärvi P, Ruuskanen J. Perspectives on the formation of polychlorinated dibenzo-p-dioxins and dibenzofurans during municipal solid waste (MSW) incineration and other combustion processes. *Acc Chem Res.* 2003; 36:652–658. [PubMed: 12974648]
- van de Ven R, Oerlemans R, van der Heijden JW, Scheffer GL, de Gruijl TD, Jansen G, Scheper RJ. ABC drug transporters and immunity: novel therapeutic targets in autoimmunity and cancer. *J Leukoc Biol.* 2009; 86:1075–1087. [PubMed: 19745159]
- Van Rechem C, Boulay G, Pinte S, Stankovic-Valentin N, Guerardel C, Leprince D. Differential regulation of HIC1 target genes by CtBP and NuRD, via an acetylation/SUMOylation switch, in quiescent versus proliferating cells. *Mol Cell Biol.* 2010; 30:4045–4059. [PubMed: 20547755]
- Van Rechem C, Rood BR, Touka M, Pinte S, Jenal M, Guerardel C, Ramsey K, Monte D, Begue A, Tschan MP, Stephan DA, Leprince D. Scavenger chemokine (CXC motif) receptor 7 (CXCR7) is a direct target gene of HIC1 (hypermethylated in cancer 1). *J Biol Chem.* 2009; 284:20927–20935. [PubMed: 19525223]
- Vorderstrasse BA, Steppan LB, Silverstone AE, Kerkvliet NI. Aryl hydrocarbon receptor-deficient mice generate normal immune responses to model antigens and are resistant to TCDD-induced immune suppression. *Toxicol Appl Pharmacol.* 2001; 171:157–164. [PubMed: 11243915]
- Wickramarachchi D, Theofilopoulos AN, Kono DH. Immune Pathology Associated with Altered Actin Cytoskeleton Regulation. *Autoimmunity.* 2010; 43(1):64–75. [PubMed: 20001423]
- Yu X, Teng H, Marques A, Ashgari F, Ibrahim SM. High resolution mapping of Cia3: a common arthritis quantitative trait loci in different species. *J Immunol.* 2009; 182:3016–3023. [PubMed: 19234197]
- Zeng XC, Luo X, Wang SX, Zhan X. Fibronectin-mediated cell spreading requires ABBA-Rac1 signaling. *J Cell Biochem.* 2013; 114:773–781. [PubMed: 23060091]
- Zhang W, Zeng X, Briggs KJ, Beaty R, Simons B, Chiu Yen RW, Tyler MA, Tsai HC, Ye Y, Gesell GS, Herman JG, Baylin SB, Watkins DN. A potential tumor suppressor role for Hic1 in breast cancer through transcriptional repression of ephrin-A1. *Oncogene.* 2010; 29:2467–2476. [PubMed: 20154726]

Highlights

Kovalova TAAP Highlights Nov. 2016

RNA-Seq identified TCDD-induced gene expression in PWM-activated primary B cells

TCDD elicited differential expression of 515 human, 2371 mouse and 712 rat orthologs

28 orthologs were differentially expressed in response to TCDD in all three species

TCDD elicits mostly species-specific gene expression changes in activated B cells

Figure 1. Number of mouse, rat and human differentially expressed genes in primary B cells exposed to 30nM TCDD

(A) Total number of differentially expressed genes up- or down-regulated at each time point in response to TCDD treatment. (B) Total number of mouse, rat and human orthologs differentially expressed at least at one time-point during the time-course.

Figure 2. Pairwise analysis of differentially expressed orthologs
Heat maps of 86 common human-mouse (A) and 12 human-rat (B) orthologs deregulated by TCDD at one or more time-points. Genes were filtered using fold change cutoff of 1.5 and, for human dataset, $P1(t) > 0.8$. White-no pDREs.

Figure 3. Functional analysis of the differentially expressed genes in the human, mouse and rat primary B cells treated with TCDD

(A) Enriched GO terms in response to TCDD treatment in primary mouse, rat and human B cells. Genes were filtered using fold change cutoff of 1.5 and, for the human dataset, $P1(t) > 0.8$ and submitted to DAVID for analysis. (B) Heat map of DEGs associated with immune response differentially expressed in response to TCDD in mouse, rat and human B cells.

Figure 4. List of KEGG biological pathways associated with genes differentially expressed in response to TCDD in primary mouse, rat and human B cells

(A) Top pathways associated with mouse, human and rat DEGs. Genes were filtered using fold change cutoff of 1.5 and, for the human dataset, $P1(t) > 0.8$ and submitted to DAVID for analysis. (B) Heat map displays differential expression of genes within common human, mouse and rat pathways in response to TCDD. Time of the highest fold-change is indicated.

Figure 5. Effect of TCDD on the PWM-induced IgM response in primary B cells

Primary human, mouse and rat B cells ($1 \times 10^5/\text{ml}$) were activated with PWM ($15 \mu\text{g}/\text{ml}$) and treated with 30 nM TCDD or vehicle (0.02% DMSO). Supernatants were harvested on day 5 (mouse rat) or 7 (human) of culture and analyzed for IgM by sandwich ELISA. Data from five human donors and five rats/mice were normalized to VH control (100%) and presented as a percentage of control. Statistical significance was determined using Dunnett's two-tailed t-test; * represents values that are significantly different from VH control at $p < 0.05$.

Figure 6. Time-dependent TCDD-elicited induction of AhR gene battery

RNA-Seq evaluation of TCDD-mediated induction of the AhR responsive genes in primary human, mouse and rat B cells activated with PWM.

Figure 7. Top implicated biological network containing common human-mouse orthologs significantly deregulated by TCDD exposure

The seed data is marked with red circles. For conventions and symbols, refer to MetaCore website legend description (https://ntp.niehs.nih.gov/ntp/ohat/diabetesobesity/wkshp/mc_legend.pdf).

Figure 8. PCR verification of the expression of select genes

Naive human B cells ($1 \times 10^6/\text{ml}$) were activated with PWM and treated with TCDD (30 nM) or VH(0.02% DMSO) for 4, 8 and 24h. Total RNA was isolated, and steady-state mRNA levels of indicated genes were measured by TaqMan real-time PCR and normalized to endogenous 18S ribosomal RNA. Data are presented as fold change compared with the VH control group. Human DEGs were validated by qRT-PCR using the same set of RNA samples used in RNA-Seq analysis plus two additional donors.

Table 1

Top ten genes differentially up- or down-regulated in primary B cells exposed to TCDD

	UP-REGULATED			DOWN-REGULATED		
	4h (Fold)	8h (Fold)	24h (Fold)	4h (Fold)	8h (Fold)	24h (Fold)
Mouse	Cyp1a1 (31.54)	Cyp1a1 (20.3)	Cyp1a1 (14.8)	Ahgef5 (-3.12)	Cldn15 (-3.15)	Sh2d1a (-3.15)
	Cyp1b1 (7.39)	Ahr (5.36)	Unc5b (5.56)	Rab13 (-3.12)	Stk30 (-3.15)	Styx (-3.15)
	Serp1b2 (5.68)	Nap1l3 (4.78)	Vdr (4.7)	Trim46 (-3.12)	Sfnbt2 (-3.15)	Evalb (-3.2)
	Ahr (4.8)	Cyp1b1 (4.39)	Efcab12 (4.7)	Zfp957 (-3.12)	Ccdc92 (-3.15)	Fasl (-3.55)
	Gpmb (4.54)	Tvp23a (3.99)	Apol10b (4.26)	Myo5b (-3.12)	Gm5814 (-3.15)	H2-B1 (-3.55)
	Sh2d1a (4.39)	Rtn4rl1 (3.91)	Pde4c (3.53)	Sirpb1c (-3.12)	Ckb (-3.15)	Gnb5 (-3.55)
	Thbs1 (4.39)	Fam229b (3.59)	Il22 (3.46)	Gm7102 (-3.12)	Spon1 (-3.15)	Cd70 (-3.95)
	Col7a1 (4.12)	Jag2 (3.59)	Cd8b1 (3.36)	Pkp2 (-3.12)	Astl (-3.15)	Msrb3 (-3.95)
	Rab4a (3.7)	Psd2 (3.59)	Trim34b (3.36)	Slc38a8 (-3.12)	Sgms2 (-3.15)	Tmem151b (-3.95)
	Smox (3.6)	Sec31b (3.59)	Mcf2l (3.36)	Tmem40 (-3.84)	Ccne1 (-2.85)	Gnaz (-3.95)
Rat	Cyp1a1 (2.71)	Acox1 (2.39)	Spred3 (1.87)	Tmem14a (-1.82)	Gstcd (-2.3)	Cebpb (-1.8)
	Pik3r3 (2.36)	Hey2 (2.19)	Slc2 (1.87)	Tbx6 (-1.82)	Fam222a (-2.45)	Igfb3 (-1.8)
	Itgb1bp2 (2.24)	Lrrc25 (2.09)	Cubn (1.75)	Cldn7 (-1.82)	Chchd10 (-2.62)	Dnlz (-1.8)
	Fcnb (2.24)	Pex19 (2.09)	Il22 (1.75)	Hhat (-1.95)	Scand1 (-2.7)	Efcab4b (-1.82)
	Rab12 (2.2)	Dna2 (1.92)	Pde4c (1.75)	Rps19 (-1.95)	Soes1 (-2.93)	Fscn3 (-1.82)
	Lst1 (2.05)	Cuedc1 (1.87)	Bried5 (1.75)	Clvs1 (-1.95)	Spr (-2.97)	Bola1 (-1.92)
	Gaml3 (1.99)	CLIC5 (1.87)	Den (1.75)	Cmklr1 (-2.07)	Rpl13 (-3.01)	Rpl13 (-1.96)
	Ifih1 (1.99)	Spred3 (1.87)	Igfb5 (1.75)	Cibp2 (-2.07)	Blocl3 (-3.36)	Card9 (-2)
	Mras (1.91)	Dyrk3 (1.87)	Prrtl (1.75)	Ppm1m (-2.09)	Nude (-3.56)	Rimbp3 (-2.16)
	Pknox1b (1.86)	Scn4a (1.84)	Pacs1n3 (1.74)	Mxd3 (-2.31)	Cox8a (-4.33)	Cox8a (-2.24)
Human	CYP1B1 (8.49)	ARL4C (4.05)	AHRR (14.24)	SLC13A3 (-2.5)	CATSPER2 (-2.9)	PEG3 (-3)
	CD93 (5.41)	C15orf26 (3.97)	CYP1A1 (10.17)	GREM1 (-2.56)	CCDC171 (-2.94)	CCNA2 (-3.02)
	STEAP4 (4.11)	ITGA1 (3.73)	ADGRB2 (5.73)	HUNK (-2.56)	SLC28A2 (-3.03)	KLC3 (-3.08)
	EREG (3.47)	KIF5A (3.67)	ASB2 (4.88)	COL15A1 (-2.56)	CD300A (-3.05)	ATP10A (-3.14)
	SLC35G5 (3.19)	BIK (3.62)	RARRES2 (4.7)	TSACC (-2.63)	XYLB (-3.12)	CACNB4 (-3.23)
	SFN (2.83)	SHISA8 (3.34)	GRIN2D (4.61)	ZC3HAV1L (-2.63)	SLC5A9 (-3.19)	UBE2OL1 (-3.25)

UP-REGULATED			DOWN-REGULATED		
4h (Fold)	8h (Fold)	24h (Fold)	4h (Fold)	8h (Fold)	24h (Fold)
SNORA5A (2.97)	ADGRB2 (3.33)	GPR68 (4.22)	MCEMP1 (-2.61)	PLEKHH2 (-3.13)	C2orf74 (-3.27)
TMPRSS9 (2.90)	MTFR2 (3.19)	PACSLN3 (4.18)	MST1 (-2.74)	FPR2 (-3.14)	DUSP15 (-3.39)
CLNK (2.87)	MYO7B (3.18)	CLCN2 (4.08)	PROKR1 (-2.81)	NRTN (-3.56)	SPNS3 (-3.61)
SHISA4 (2.78)	SATB2 (3.15)	ADAM11 (4.04)	SLC16A14 (-3)	TAS2R4 (3.62)	TMEM40 (-4.04)

Table 2
Conserved, TCDD-Induced Ortholog Differential Expression

The time point of the maximum fold change is indicated in brackets. Mouse, rat and human data sets were filtered using |fold change| cutoff of 1.5 and for human dataset, $P1(t) > 0.8$. High pDRE have an MSS = 0.8 while Very High pDRE have an MSS = 0.9.

GENE Symbol	HID	HUMAN	MOUSE	RAT
AHRR	49215	14.2 (24)	5.3 (8)	1.8 (4)
CD27	74386	2.8 (8)	1.4 (24)	1.4 (24)
CYP1A1	68062	10.2 (24)	31.5 (4)	2.7 (4)
MTSS1L	70926	2.0 (8)	1.9 (24)	1.6 (4)
MYO6	56417	2.3 (24)	1.9 (8)	1.5 (8)
SLC24A3	41390	1.9 (4)	1.9 (24)	1.9 (4)
TIPARP	9167	2.1 (24)	3.0 (4)	1.6 (4)
CABYR	49299	-2 (8)	-2 (8)	-1.5 (24)
CHRM4	20192	-3.3 (8)	-2 (8)	-1.7 (24)
P2RY13	12543	-2 (4)	-1.7 (4)	-1.7 (4)
HIC1	4740	2.2 (8)	2.2 (4)	-2 (8)
ITGB3	55444	1.9 (24)	1.5 (8)	-2 (24)
BEND7	17660	2.4 (8)	1.8 (4)	-1.7 (4)
DLG5	3486	2.1 (24)	1.8 (8)	-1.7 (4)
PLXDC2	41666	2.3 (8)	1.8 (8)	-1.7 (24)
PRR18	52195	2.3 (24)	2.1 (4)	-1.7 (8)
RND2	21123	1.6 (4)	1.5 (4)	-1.7 (8)
SHF	44524	1.7 (8)	1.5 (24)	-1.7 (4)
STAP2	9798	1.8 (24)	1.8 (8)	-2 (8)
FSCN2	22722	2.1 (24)	-2 (4)	1.5 (4)
REEP2	41146	2.5 (24)	-2 (8)	1.5 (24)
PACSIN3	41117	4.1 (24)	-2 (4)	1.7 (24)
SEMA6B	8428	2.7 (4)	-1.7 (24)	1.6 (24)
HEY2	22705	-3.3 (24)	1.8 (4)	2.2 (8)
MAP6	7850	-2 (24)	1.8 (8)	1.7 (4)
GPR160	8659	1.7 (8)	-1.7 (24)	-2 (8)
PHLDA1	7203	1.6 (24)	-1.7 (8)	-2 (8)
TOX2	13155	-1.7 (8)	1.8 (8)	-2 (4)

 ■ pDRE (Very High)

 ■ pDRE (High)

Table 3
Gene ontology analysis of the 86 common human-mouse DEGs

Genes were filtered using fold change cutoff of 1.5 and, for the human dataset, $P1(t) > 0.8$.

GO ID	GO Term	Fisher p-value
GO:0031012	extracellular matrix	1.50E-04
GO:0007229	Integrin-mediated signaling pathway	3.30E-04
GO:0005509	calcium ion binding	7.40E-04
GO:0043394	proteoglycan binding	1.50E-03
GO:0005178	integrin binding	2.40E-03
GO:0005578	proteinaceous extracellular matrix	3.20E-03
GO:0007155	cell adhesion	5.40E-03
GO:0008201	heparin binding	1.10E-02
GO:0005518	collagen binding	1.20E-02
GO:0001948	glycoprotein binding	1.20E-02
GO:0044420	extracellular matrix part	1.60E-02
GO:0030246	carbohydrate binding	2.20E-02
GO:0005539	glycosaminoglycan binding	2.60E-02
GO:0030247	polysaccharide binding	3.30E-02
GO:0001871	pattern binding	3.30E-02
GO:0032989	cellular component morphogenesis	3.90E-02
GO:0032403	protein complex binding	6.00E-02
GO:0009986	cell surface	7.30E-02
GO:0043066	anti-apoptosis	7.40E-02
GO:0005871	kinesin complex	7.90E-02
GO:0007050	cell cycle arrest	8.50E-02
GO:0009968	negative regulation of signal transduction	8.70E-02
GO:0019200	carbohydrate kinase activity	8.70E-02
GO:0000902	cell morphogenesis	8.80E-02
GO:0030141	secretory granule	2.00E-01