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The Aryl Hydrocarbon Receptor Regulates an Essential Transcriptional Element in the Immunoglobulin Heavy Chain Gene

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Abstract

Ig heavy chain (*Igh*) transcription involves several regulatory elements including the 3'*Igh* regulatory region (3'*IghRR*). 3'*IghRR* activity is modulated by several transcription factors, including NF- κ B and AP-1 and potentially the aryl hydrocarbon receptor (AhR). The prototypical AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits antibody secretion and 3'*IghRR* activity. However, the exact mechanism is unknown and TCDD can modulate NF- κ B and AP-1 in an AhR-independent manner. To determine if the AhR is a significant regulator of the 3'*IghRR*, we utilized a mouse B-cell line that stably expresses a 3'*IghRR*-regulated transgene and either an AhR antagonist or shRNA targeting the AhR. Disruption of the AhR pathway reversed TCDD-induced suppression of the 3'*IghRR*-regulated transgene and of endogenous Ig demonstrating a biologically significant effect of the AhR on 3'*IghRR* activation. Altered human 3'*IGHRR* activity by AhR ligands, which include dietary, environmental, and pharmaceutical chemicals, may have significant implications to human diseases previously associated with the 3'*IGHRR*.

Keywords

aryl hydrocarbon receptor; B cells; dioxin; gene regulation; 3'*Igh* regulatory region; immunoglobulin expression; immunoglobulin heavy chain; immunosuppression; TCDD; transcriptional enhancers

1. INTRODUCTION

Of the Ig genes, regulation of the mouse *Igh* locus is the best understood and is achieved through a variable heavy chain promoter (V_H)¹, an intronic enhancer (E_μ), and the 3'*Igh* regulatory region (3'*IghRR*). Primarily activated in terminally differentiated antibody-secreting cells, the 3'*IghRR* provides for high *Igh* expression as well as class-switch

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recombination [1]. In humans, the 3'*IgHRR* has been associated with several autoimmune diseases and certain B-cell lymphomas [2–7]. Additionally, we have previously identified the mouse 3'*IghRR* as a sensitive target of exogenous compounds including dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is a ubiquitous environmental contaminant and the prototypical high affinity ligand for the nuclear receptor and transcription factor known as the aryl hydrocarbon receptor (AhR). Canonically, the AhR is known to induce the transcription of xenobiotic metabolizing enzymes such as cytochrome P450s (e.g. Cyp1A1), but increasingly, evidence supports AhR regulation of genes involved in physiological processes including cellular proliferation and differentiation [8,9].

Several studies utilizing various animal models have established a TCDD-induced inhibition of B-lymphocyte differentiation into antibody-forming cells and support a role of the AhR in this inhibitory effect [10]. Ig heavy and light chain (*Igh* and *Igl*, respectively) gene expression appears to be inhibited by TCDD since previous studies have demonstrated an inhibition of *Igh* and *Igl* mRNA levels that correlates with a decrease in antibody levels [11–13]. These effects could be mediated by decreased stability of mRNA transcripts or a direct effect on transcriptional regulatory elements within the *Igh* and *Igl* genes. In support of a transcriptional effect, chemical-induced modulation of 3'*IghRR* activity by AhR ligands – as well as non-AhR ligands – mirrored the effects on Ig protein levels [14]. Therefore, exposure to environmental triggers may functionally alter 3'*IghRR* activity leading to altered Ig levels and humoral immunity.

The mouse 3'*IghRR* contains at least four DNase I hypersensitivity sites (hs3A, hs1.2, hs3B, and hs4), which exhibit enhancer activity and contain several transcription factor binding sites, such as NF-κB, AP-1, Oct, and Pax-5 [1,15]. A novel binding site for the AhR (i.e. dioxin responsive element or DRE) was identified in both the mouse hs1.2 and hs4 enhancers suggesting a potential regulatory role of the AhR in 3'*IghRR* activation [11]. However, TCDD can modulate DNA binding and/or transcriptional activity of transcription factors such as NF-κB/Rel and AP-1 in an AhR-independent manner [11,16]. Since a plethora of transcription factors, including NF-κB/Rel and AP-1, control 3'*IghRR* (reviewed in [1]), the objective of the current study was to determine if the AhR is a significant biological regulator of the 3'*IghRR*. To achieve this objective, we utilized an Ig-expressing mouse B-cell line (CH12.LX) that has a stable insert of a previously characterized 3'*IghRR*-regulated transgene [14] and either pharmacological inhibition of the AhR or shRNA knockdown of AhR expression.

Disruption of the AhR signaling pathway reversed the inhibition by TCDD of the 3'*IghRR*-regulated transgene, which functionally correlated with the biological effects on endogenous Ig expression. These results address a critical mechanistic gap and identify the AhR as a significant and dominant regulator of the 3'*IghRR* that at least partially mediates TCDD-

¹The Abbreviations used are: AhR, aryl hydrocarbon receptor; AhRA, AhR antagonist (CH-223191); ARNT, AhR nuclear translocator; bHLH, basic-helix-loop-helix; CH12.γ2b-3'*IghRR*, CH12.LX cells stably expressing a 3'*IghRR*-regulated γ2b transgene; C, LPS alone control; Cyp1A1, cytochrome P4501A1; DMSO, dimethyl sulfoxide; DRE, dioxin response element; Eμ, intronic enhancer; FP, forward primer; IgH, Ig heavy chain; 3'*IghRR*, mouse 3'*Igh* regulatory region; 3'*IgHRR*, human 3'*IgH* regulatory region; IgL, Ig light chain; IS, invariant sequence; LPS, lipopolysaccharide; NA, naïve control; RP, reverse primer; RQ, relative quantitation; shAhR, shRNA against AhR mRNA; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; V_H, variable Ig heavy chain promoter; WT, wild type

induced inhibition of Ig by inhibiting the capacity of the 3'*Igh*RR to increase *Igh* expression. Additionally, TCDD represents a large class of polychlorinated dibenzodioxins, dibenzofurans, and biphenyls that have been the prototypical ligands by which the AhR signaling pathway has been primarily characterized. However, a variety of nondioxin agonists have been recently discovered including those of pharmaceutical and dietary origins [17–19]. Moreover, the human 3'*IGH*RR has been associated with diseases such as Burkitt's lymphoma and several autoimmune diseases including celiac disease, IgA nephropathy, systemic sclerosis, dermatitis herpetiformis, plaque psoriasis, psoriatic arthritis, and rheumatoid arthritis [2–7]. Therefore, modulation of the human 3'*IGH*RR by environmentally ubiquitous dioxin and nondioxin AhR ligands has the potential to influence the severity and/or incidence of human diseases associated with the 3'*IGH*RR.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

AhR antagonist (CH-223191) was purchased from EMD4Biosciences (Newark, NJ) at > 95% purity and suspended in 100% dimethyl sulfoxide (DMSO). TCDD (99.1% purity) in 100% DMSO was purchased from Accustandard (New Haven, CT). DMSO and lipopolysaccharide (LPS, *Escherichia coli*) were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 Cell Lines

The CH12.γ2b-3'*Igh*RR cell line, developed by our lab [14], is a variant of the CH12.LX B-cell line derived from the CH12 B-cell lymphoma, which arose in B10.H-2^aH-4^bp/Wts mice [20]. The CH12.γ2b-3'*Igh*RR cell line endogenously expresses IgA and stably expresses a 3'*Igh*RR-regulated γ2b-transgene mini-locus. The γ2b mini-locus was previously characterized and generously provided by Dr. Laurel Eckhardt from Hunter College, New York, NY [21]. PCR and ELISA analysis verified that the CH12.γ2b-3'*Igh*RR cell line does not endogenously express γ2b and that the γ2b-transgene is activated by LPS with maximal expression at 48 hr [14].

Cells were grown in a 37°C incubator with 5% CO₂ injection. Cells were maintained in RPMI 1640 media (Mediatech, Herndon, VA) supplemented with 10% bovine calf serum (Hyclone Laboratories, Logan, UT), 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 13.5 mM HEPES, 100 units/ml penicillin, 50 μM 2-mercaptoethanol, and 100 μg/ml streptomycin (Hyclone Laboratories).

2.3 shRNA Constructs

Two pLKO.1 HIV-based lentiviral vector plasmids containing shRNA sequences complimentary to AhR (shAhR) and puromycin-selectable marker gene were purchased from Open Biosystems (Huntsville, AL). The shAhR sequences target nucleotides in the AhR transcript (Genbank Accession No. NM_013464.4) as follows: shAhR11, 5'-AATTT GCTCATGTTTCAGCGC-3', corresponding to nucleotide positions 1861-1881 and shAhR12, 5'-TAATAACATCTTGCGGGAAGG-3', corresponding to nucleotide positions 527-547. Vectors were packaged into VSV-G pseudotyped fourth generation lentiviral

particles by Cincinnati Children's Viral Vector Core (Cincinnati, OH) and stored at -80°C until use.

2.4 Stable AhR Knockdown Cells

CH12. γ 2b-3'*IghRR* cells (5×10^3 cells/ml) were resuspended in media containing 16 $\mu\text{g/ml}$ polybrene (American Bioanalytical, Natick, MA) then seeded into a 96-well, flat-bottom culture plate (500 cells in 0.1 ml media per well) and 100 μl of either shAhR11 or shAhR12-containing lentiviral particles was added (8 $\mu\text{g/ml}$ final concentration of polybrene). Culture plates were immediately centrifuged for 30 minutes at $1100 \times g$ before supernatants were discarded and replaced with 200 μL fresh media. After 24 hr, cell supernatant was replaced with puromycin (Invivogen, San Diego, CA)-selective media (1 $\mu\text{g/ml}$). Puromycin-selective media was replaced every 72 hr for approximately 4 weeks until cell density and culture volumes were sufficient to harvest whole cell lysate from 10 ml of cells and 1.0×10^6 cell stocks were frozen in liquid nitrogen for future use.

Cell cultures were strictly maintained to avoid a high passage number during any culture period and were never allowed to over grow or plateau in growth. A stable empty vector was not generated for these studies because the vector can be inserted in different places in the genome and would not truly control for our shAhR stables. Additionally, studies have demonstrated a lack of usefulness of scrambled siRNA in controlling for siRNA experiments [22,23]. Rescuing the phenotype is the best control for siRNA experiments but is technically infeasible in cell types, including those used in the current study, that have low transfection efficiency and therefore do not sufficiently express exogenous cDNA.

Alternatively, using more than one shRNA target for the gene of interest will provide a confirmation of specificity [22]. Corresponding, two different siRNA targets for the AhR (i.e. shAhR11 vs. shAhR12) were utilized as described above. In addition, two different methods to disrupt AhR signaling (i.e. shAhR vs. chemical antagonist) were employed and both resulted in the same outcome regardless of the method or the shAhR construct used. Furthermore, there was no impairment of the ability to induce 3'*IghRR* activation or Ig expression in our shAhR cells following LPS stimulation, supporting a normal B-cell function.

2.5 Chemical Treatment

Cells were treated with the AhR antagonist, CH-223191, or vehicle (0.1% DMSO) for one hour prior to treatment with TCDD (10 nM) or the appropriate TCDD vehicle (0.01% DMSO). For IgA and γ 2b analysis, cells were stimulated with LPS (1 $\mu\text{g/ml}$), seeded in 24-well culture plates at a concentration of 3.0×10^4 cells/ml and incubated for 48 hours at 37°C in 5% CO_2 . For quantitative real-time PCR of Cyp1A1 transcripts, cells were seeded at 5.0×10^5 cells/ml in 24-well culture plates and incubated for 8 h. No consistent vehicle effects were seen (i.e. no increase in Cyp1A1 transcripts).

2.6 Protein Isolation and Western Blot

Cells were centrifuged at 3000 rpm then lysed with mild lysis buffer (1% NP-40, 150 mM NaCl, 2 mM EDTA, 10 mM NaPO_4) containing protease inhibitors (Complete Mini Protease Inhibitor Cocktail; Roche, Indianapolis, IN) and frozen at -80°C . Protein

quantification was performed by thawing whole cell lysates on ice prior to centrifugation at 14,000 rpm. Supernatants were collected and protein content quantified by a Bio-Rad Assay (Hercules, CA). Protein samples were normalized to 2 μ g in 100 μ L of 1x PBS in preparation for ELISA analysis. Naive samples were also used for SDS-PAGE and Western blotting analysis of AhR protein expression. Briefly, 20 μ g of total protein was denatured then electrophoresed on a 10% polyacrylamide gel at 200 volts for ~40 min. Proteins were transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA) at 100 volts for 1 h. Membranes were blocked for 1 h in TBS with 3% BSA and 0.05% tween-20 then incubated with either mouse anti-AhR (Ab2770, Abcam, Cambridge, MA) at a 1:1000 dilution or mouse anti- β -actin (Sigma-Aldrich) at a 1:10,000 dilution in TBS with 1% BSA and 0.05% tween-20. Membranes were washed four times in TBS with 0.05% tween-20 before incubating for 1 h with HRP-conjugated goat anti-mouse (Santa Cruz) at a 1:2500 dilution in TBS with 0.05% tween-20. All incubations were performed at room temperature. Proteins were detected using Pierce Supersignal substrate (Thermoscientific Pierce, Waltham, MA) in a Fuji LAS-3000 Bioimager (Tokyo, Japan) at 30 second intervals.

2.7 ELISA

Concentrations of IgA and γ 2b in cell lysates were analyzed as described previously [14]. Colorimetric detection was performed every minute for 1 hour using a Spectramax Plus 284 automated microplate reader with a 405-nm filter (Molecular Devices, Sunnyvale, CA). Sample concentrations of IgA and γ 2b were calculated by the SOFTmax PRO analysis software (Molecular Devices) using a standard curve generated from the kinetic rate of the absorption for known IgA or γ 2b concentrations. IgA and γ 2b expression are shown as both ng/2 μ g total protein and as a percent of the DMSO vehicle control (set to 100%).

2.8 RNA Isolation and cDNA Synthesis

After the incubation period, cells were centrifuged at 3000 rpm, collected in 0.25 ml of TRI Reagent (Sigma-Aldrich), and stored at -80°C . RNA was isolated as before [24]. Briefly, samples were thawed at room temperature, mixed with 0.1 volume of 1-bromo-3-chloropropane, and the aqueous phase separated using Phase Lock Gel Heavy Tubes (5 PRIME, Gaithersburg, MD). RNA was precipitated using isopropanol and washed with 75% ethanol before suspension in nuclease-free H_2O and storage at -20°C until analysis.

RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE). One microgram of total RNA was converted to cDNA using the TaqMan Reverse Transcriptase Kit (ABI, Carlsbad, CA) following the standard manufacturer's protocol. The cycling conditions for reverse transcription were as follows, 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min.

2.9 Real-Time PCR

TCDD-induced Cyp1A1 expression in wild type, CH223191-treated, and shAhR cell lines was determined by SYBR Green real-time PCR. Cyp1A1 and β -actin (endogenous control) transcripts were amplified from 5 ng cDNA utilizing the following primers: Cyp1A1 forward primer (FP), 5' AAGTGCAGATGCGGTCTTCT 3'; Cyp1A1 reverse primer (RP), 5' AAAGTAGGAGGCAGGCACAA 3'; β -actin FP, 5'-

GCTACAGCTTCACCACCACA-3'; β -actin RP, 5'-TCTCCAGGGAGGAAGAGGAT-3'. cDNA was combined with 6 pmol of both FP and RP, 2x SYBR Green Master Mix (Applied Biosystems), and diluted to 25 μ L with nuclease-free water. Separate real-time PCR reactions were performed for Cyp1A1 and β -actin using an ABI 7500 with cycling conditions of 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. Relative Quantification (RQ) values (i.e. fold-change) were determined by the ABI 7500 SDS 2.0. Because Cyp1A1 has little to no expression in the absence of an AhR ligand (i.e. naïve and vehicle-treated cells), Cyp1A1 expression is presented as a fold-change (RQ) relative to the Cyp1A1 expression in wild type (WT) cells treated with 10 nM TCDD (set to 1).

2.10 Statistical Analysis

The mean $\pm$ SE (n=4) was calculated for all treatment groups for each experiment. Data shown is representative of three to four separate experiments. Significance of treatments from controls was determined by a 1-way ANOVA and Dunnett's post-hoc test for the endogenous IgA or γ 2b transgene proteins and a one-tailed t-test for Cyp1A1 transcripts.

3. RESULTS

3.1 Disruption of canonical AhR signaling in a mouse B-lymphocyte cell line

Using the well-characterized CH12.LX B-lymphocyte cell line model, previous studies have supported a role of the AhR and the 3'*Igh*RR in the inhibition of Ig expression by TCDD [11,14,25,26]. However, it is unknown whether TCDD modulates 3'*Igh*RR activity directly through the AhR. Underscoring this uncertainty, TCDD may alter the binding and/or activity of several other transcription factors (i.e. NF- κ B, AP-1, Oct) thought to be involved in 3'*Igh*RR activity and Ig expression [1,13,15,26–32]. To directly evaluate the role of the AhR in the effects of TCDD on the 3'*Igh*RR and Ig, we utilized either shRNA against the AhR or an AhR antagonist (CH223191) to inhibit the AhR signaling pathway in an IgA-expressing variant of the CH12.LX cell line that stably expresses a γ 2b transgene regulated by the 3'*Igh*RR (CH12. γ 2b-3'*Igh*RR cells) [14]. For shRNA knockdown of AhR expression, CH12. γ 2b-3'*Igh*RR cells were stably transduced with lentiviral particles containing one of two independent shRNA sequences (denoted shAhR11 or shAhR12) targeted to the AhR. AhR protein levels were stably knocked down by at least 50% by shAhR11 and greater than 50% by shAhR12 (Fig. 1A). To verify a functional effect due to this AhR knockdown, we utilized real-time PCR to measure the hallmark response of AhR activation, i.e. induction of Cyp1A1 expression [33]. As we have previously demonstrated in the parental CH12.LX cells, TCDD induced a marked increase in Cyp1A1 expression (data not shown) [11,25]. Pre-treatment of the CH12. γ 2b-3'*Igh*RR cells with the AhR antagonist CH223191 [34,35] resulted in a nearly 6-fold decrease in TCDD-induced Cyp1A1 expression (Fig. 1B). Similar to the pharmacological effects of the AhR antagonist, Cyp1A1 induction by TCDD was repressed in the shAhR12 and shAhR11 cell populations by five-fold and two-fold, respectively, compared to that of the wild type CH12. γ 2b-3'*Igh*RR cells (Fig. 1B).

3.2 Disruption of AhR signaling reverses TCDD-induced inhibition of 3'IghRR activation and Ig expression

The AhR has long been suspected to mediate TCDD-induced inhibition of antibody secretion and Ig expression, as supported by structure-activity relationships, congenic mouse models, and AhR-null models (mouse or B-lymphocyte cell line) [10]. However, there remains a paucity of mechanistic data to explain how the AhR regulates Ig expression. Our lab has previously shown that a key regulator of *Igh* transcription and therefore Ig production, the 3'*IghRR*, not only contains DRE sites that bind the AhR/ARNT, but is also a sensitive transcriptional target of TCDD [11,13,14]. Despite this link, the role of the AhR in 3'*IghRR* activity has not been directly established. Therefore, we sought to elucidate the relationship between the AhR and the 3'*IghRR* using the competitive AhR antagonist and the shAhR cell populations (shAhR11 and shAhR12) characterized above.

Since our cellular model (CH12.γ2b-3'*IghRR*) stably expresses a γ2b transgene regulated by the 3'*IghRR*, the AhR-dependent changes in 3'*IghRR* transcriptional activity and endogenous Ig expression can be simultaneously evaluated. Consistent with our previous studies, LPS-stimulation induced protein expression of both the γ2b transgene and endogenous IgA in the wild-type (CH12.γ2b-3'*IghRR*) and shAhR cell populations (Figs. 2 and 3) [14], supporting normal B-cell function and intact LPS-induced cellular signaling within the shAhR cells. TCDD treatment inhibited γ2b and IgA in the wild-type cells, which was completely abolished by a one-hour pretreatment with the AhR antagonist (Fig. 2). In addition, LPS-induced expression of γ2b and IgA in the shAhR11 and shAhR12 cell populations was refractory to TCDD compared to the wild type cells (Fig. 3). Moreover, decreased AhR expression or function did not appear to alter basal or induced 3'*IghRR* activity or Ig expression (Figs. 2 and 3). Taken together, the ligand-activated AhR appears to be a dominant negative regulator of 3'*IghRR* activation.

4. DISCUSSION

Igs are essential for maintaining immunity against a wide variety of pathogens but Igs can also play a role in pathogenic states, such as hypersensitivity reactions and autoimmune diseases. Therefore altered Ig expression could have significant consequences to human health. However, the mechanisms for Ig dysregulation are far from clear and likely involve genetic susceptibility and environmental exposures to immune altering xenobiotics. In mouse models, the transcriptional regulatory region, 3'*IghRR*, located downstream of the *Igh* locus plays a major role in *Igh* expression and class switch recombination and is sensitive to chemical-induced modulation [1,14,26]. In support of a biologically relevant effect, chemical-induced modulation of 3'*IghRR* activity mirrored the effects on Ig protein levels [14]. Therefore, exposure to environmental triggers may functionally alter 3'*IghRR* activity leading to altered Ig levels and potential pathological effects.

A significant chemical modulator of 3'*IghRR* activity is TCDD, a well-known and potent suppressor of Ig expression and the antibody-forming cell response in laboratory animals [10]. TCDD is a high affinity ligand for the AhR, which is a nuclear receptor and transcription factor. The AhR, when activated by ligand, plays a prominent role in upregulating metabolic enzymes but the endogenous role of the AhR is less clear. This is a

significant human health question given the plethora of nondioxin AhR ligands of pharmaceutical and dietary origin [36,37]. Indeed, the limited number of studies with human B lymphocytes has demonstrated altered Ig expression by TCDD [38–40], suggesting the potential of AhR ligands in general to alter B-lymphocyte function in humans. Elucidating the role of the AhR in regulating 3'*Igh*RR activity and Ig expression is a critical component in assessing risk to human health as well as offering the potential to develop new pharmacological interventions for diseases exhibiting a significant antibody component.

The mouse 3'*Igh*RR is a large ~40 kb region that contains at least four enhancers (i.e. hs3A, hs1.2; hs3B; hs4) with binding sites for many transcription factors [1,15], including the AhR [11]. These transcription factors likely mediate a complex interaction between the 3'*Igh*RR enhancers, V_H promoter, and transcriptional machinery [41,42]. Through both pharmacological inhibition and shRNA knockdown, the current study is the first to demonstrate a dominant regulatory role of the AhR in 3'*Igh*RR activity. These results support a mechanism in which a ligand-activated AhR inhibits the capacity of the 3'*Igh*RR to increase *Igh* transcription, therefore leading to suppressed Ig levels. A single enhancer of the 3'*Igh*RR or a combination of these enhancers may direct the overall inhibition of the mouse 3'*Igh*RR by TCDD. Relevant to this notion is the striking difference in transcriptional behavior exhibited by the hs1.2 and hs4 enhancers when studied in isolation. Both contain several transcription factor binding sites including a DRE motif capable of binding the AhR [11,43]. However, the hs4 enhancer is synergistically activated by TCDD and LPS treatment; while similar to the 3'*Igh*RR, LPS-induced transcriptional activation of the hs1.2 enhancer is inhibited by TCDD [13,26,27]. This dichotomy in regulation of hs4 and hs1.2 enhancer activity was also demonstrated in mature B-cell lines with NF-κB, Oct, and Pax-5, in that these transcription factors negatively influenced hs1.2 activity but positively influenced hs4 activity [43]. Interestingly, the human hs1.2 enhancer contains a putative AhR binding site and is sensitive to TCDD, but in marked contrast to the inhibitory effect of TCDD on the mouse hs1.2 enhancer, the human hs1.2 enhancer is activated by TCDD [13]. Although there is a fairly high degree of sequence similarity between the human and mouse hs1.2 enhancer, including transcription factor binding sites, there is not 100% conservation (e.g. lack of Pax-5 sites in human hs1.2), which may account for the species difference in the effects of TCDD on hs1.2 activity [13,44,45].

Clearly, predicting the behavior (i.e. inhibition vs. activation) of AhR-sensitive genes cannot be restricted to the simple identification of DRE motifs and must be considered in the context of the entire transcriptional unit and the interactions between various transcription factors. Notably, TCDD and the AhR signaling pathway influence DNA binding and/or transcriptional activity of several transcription factors thought to mediate mouse 3'*Igh*RR activity and Ig expression (i.e. NF-κB, Pax-5, AP-1, and Oct) [16,28–32,43,46,47]. Furthermore, TCDD can modulate NF-κB/Rel and AP-1 in an AhR-independent manner [11,16]. Therefore a complex and differing interaction between many transcription factors likely mediates the effects of LPS stimulation and of TCDD on 3'*Igh*RR activation and ultimately Ig expression. The extent or composition of these interactions probably varies depending on maturation state, stimulation conditions, and/or exogenous exposures. Regardless of the probable multi-factor interplay between specific transcription factors

binding within the 3'/IghRR, the results of the current study has identified the AhR as a dominant negative regulator of 3'/IghRR activation. Alternatively, loss of the AhR does not appear to play a role in basal or LPS-stimulated 3'/IghRR activity and TCDD in the absence of the AhR does not appear to modulate other transcription factors, such as NF- κ B/Rel or AP-1, to an extent to cause an observable effect on 3'/IghRR activity or Ig expression.

Besides the differences in the hs1.2 enhancer, the structure of the human 3'/IGHRR diverges slightly from that of the mouse 3'/IghRR complicating translation of mouse 3'/IghRR studies to the human 3'/IghRR. In the human IGH locus, the 3'/IGHRR is duplicated and consists of only three enhancers (hs3, hs1.2, and hs4) [44,45]. Additionally, the human hs1.2 enhancer is polymorphic as characterized by an approximately 38 bp invariant sequence that may be repeated in tandem up to three times [48–50]. Interestingly, the human hs1.2 polymorphism has been correlated with the incidence of many immune-related diseases including celiac disease, IgA nephropathy, systemic sclerosis, plaque psoriasis, psoriatic arthritis, dermatitis herpetiformis, rheumatoid arthritis, and systemic lupus erythematosus [3–7,51]. Significantly, the invariant sequence contains a DRE-like site and studies using the AhR antagonist CH223191 revealed that human hs1.2 enhancers containing one, two, or three copies of the invariant sequence are activated by TCDD in an AhR-dependent manner and in general with increasing reporter activity with an increased number of invariant sequences [13]. Therefore, the AhR signaling pathway may influence the diseases associated with the polymorphic hs1.2 enhancer. However, these studies were performed using transiently expressed reporters solely regulated by the hs1.2 enhancer and may not be reflective of the full human 3'/IGHRR.

To our knowledge the physiological role of the full human 3'/IGHRR has not been characterized and very few studies have evaluated the effect of TCDD on human B-lymphocyte function and Ig expression. Results of these limited studies appear to support a variation in the magnitude of the response (i.e. altered IgM expression) of human primary tonsillar lymphocytes and primary peripheral blood B lymphocytes to TCDD with the most responsive cells demonstrating a similar sensitivity to TCDD as highly responsive mouse models, i.e. strains such as C57BL/6 that carry the high affinity AhR^b allele [38,40]. However, the human AhR has an approximately 10-fold lower affinity for TCDD compared to the mouse AhR^b allele due to a nucleotide difference (A375V) within the ligand binding region [52]. Therefore the similar sensitivity of mouse and human IgM expression to TCDD suggests a more complicated mechanism than just simple transcriptional regulation through AhR-DRE binding and may include altered activity of several transcription factors (i.e. NF- κ B, AP-1, and Oct) and the 3'/IGHRR enhancers as alluded to above. Interestingly, in one study the actual effect of TCDD on the human IgM response varied, demonstrating an inhibitory effect in most donor cells and no effect or an increase in the IgM response in other donor cells, which may relate to differences in AhR function perhaps due to the SNPs identified in the AhR [40,53]. Alternatively, these differences may be related to the polymorphic hs1.2 enhancer, which was not evaluated. In a different study, TCDD induced an increase in spontaneous IgE production in B cells from atopic patients with allergic rhinitis, atopic eczema/dermatitis syndrome, or bronchial asthma but not healthy controls [39]. These limited studies support a complex effect of TCDD on Ig responses in human B

lymphocytes, which likely involves the influence of many factors, including AhR function, disease state, and perhaps the hs1.2 polymorphism and altered 3'*IGHRR* activity.

5. Conclusions

The current study is the first to demonstrate a dominant role of the AhR in regulating mouse 3'*IghRR* activity, which appears to mediate the inhibitory effect of TCDD on Ig levels. Therefore our study fills a critical mechanistic data gap and also underscores the necessity of elucidating the role of the human 3'*IGHRR* in Ig expression and sensitivity to AhR ligands. Additionally, the association of the hs1.2 polymorphism to several immune-related diseases [3–7,51] suggests that the growing number of dietary and pharmaceutical substances with affinity for the AhR and the persistent presence of dioxin-related compounds in the environment may have the capacity to alter the incidence and/or the severity of human diseases associated with the polymorphic hs1.2 enhancer [36,37]. Understanding the role of the AhR in the transcriptional activity of the 3'*IghRR* would provide valuable insight into the identification and quantification of human health risks and may provide opportunities for therapeutic intervention in many immune-related disorders.

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References

1. Pinaud E, Marquet M, Fiancette R, Peron S, Vincent-Fabert C, Denizot Y, Cogne M. The IgH locus 3' regulatory region: pulling the strings from behind. *Adv Immunol.* 2011; 110:27–70. [PubMed: 21762815]
2. Wang J, Boxer LM. Regulatory elements in the immunoglobulin heavy chain gene 3'-enhancers induce c-myc deregulation and lymphomagenesis in murine B cells. *J Biol Chem.* 2005; 280:12766–12773. [PubMed: 15687498]
3. Aupetit C, Drouet M, Pinaud E, Denizot Y, Aldigier JC, Bridoux F, Cogne M. Alleles of the alpha1 immunoglobulin gene 3' enhancer control evolution of IgA nephropathy toward renal failure. *Kidney Int.* 2000; 58:966–971. [PubMed: 10972660]
4. Frezza D, Giambra V, Cianci R, Fruscalzo A, Giufre M, Cammarota G, Martinez-Labarga C, Rickards O, Scibilia G, Sferlazzas C, Bartolozzi F, Starnino S, Magazzu G, Gasbarrini GB, Pandolfi F. Increased frequency of the immunoglobulin enhancer HS1,2 allele 2 in coeliac disease. *Scand J Gastroenterol.* 2004; 39:1083–1087. [PubMed: 15545166]
5. Frezza D, Giambra V, Tolusso B, De Santis M, Bosello S, Vettori S, Triolo G, Valentini G, Ferraccioli G. Polymorphism of immunoglobulin enhancer element HS1,2A: allele *2 associates with systemic sclerosis. Comparison with HLA-DR and DQ allele frequency. *Ann Rheum Dis.* 2007; 66:1210–1215. [PubMed: 17392350]

6. Cianci R, Giambra V, Mattioli C, Esposito M, Cammarota G, Scibilia G, Magazzu G, Orlando A, Sandri G, Bianchi L, Gasbarrini GB, Pandolfi F, Frezza D. Increased frequency of Ig heavy-chain HS1,2-A enhancer *2 allele in dermatitis herpetiformis, plaque psoriasis, and psoriatic arthritis. *J Invest Dermatol.* 2008; 128:1920–1924. [PubMed: 18323783]
7. Tolusso B, Frezza D, Mattioli C, Fedele AL, Bosello S, Faustini F, Peluso G, Giambra V, Pietrapertosa D, Morelli A, Gremese E, De Santis M, Ferraccioli GF. Allele *2 of the HS1,2A enhancer of the Ig regulatory region associates with rheumatoid arthritis. *Ann Rheum Dis.* 2009; 68:416–419. [PubMed: 18952640]
8. Ma C, Marlowe JL, Puga A. The aryl hydrocarbon receptor at the crossroads of multiple signaling pathways. *EXS.* 2009; 99:231–257. [PubMed: 19157064]
9. Abel J, Haarmann-Stemmann T. An introduction to the molecular basics of aryl hydrocarbon receptor biology. *Biol Chem.* 2010; 391:1235–1248. [PubMed: 20868221]
10. Sulentic CEW, 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]
11. Sulentic CEW, Holsapple MP, Kaminski NE. Putative link between transcriptional regulation of IgM expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin and the aryl hydrocarbon receptor/dioxin-responsive enhancer signaling pathway. *J Pharmacol Exp Ther.* 2000; 295:705–716. [PubMed: 11046109]
12. Yoo BS, Boverhof DR, Shnaider D, Crawford RB, Zacharewski TR, Kaminski NE. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) alters the regulation of Pax5 in lipopolysaccharide-activated B cells. *Toxicol Sci.* 2004; 77:272–279. [PubMed: 14600275]
13. Fernando TM, Ochs SD, Liu J, Chambers-Turner RC, Sulentic CEW. 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Induces Transcriptional Activity of the Human Polymorphic hs1,2 Enhancer of the 3'IgH Regulatory Region. *J Immunol.* 2012; 188:3294–3306. [PubMed: 22357631]
14. Henseler RA, Romer EJ, Sulentic CEW. Diverse chemicals including aryl hydrocarbon receptor ligands modulate transcriptional activity of the 3'immunoglobulin heavy chain regulatory region. *Toxicology.* 2009; 261:9–18. [PubMed: 19447539]
15. Khamlichi AA, Pinaud E, Decourt C, Chauveau C, Cogne M. The 3' IgH regulatory region: a complex structure in a search for a function. *Adv Immunol.* 2000; 75:317–345. [PubMed: 10879288]
16. Hoffer A, Chang CY, Puga A. Dioxin induces transcription of fos and jun genes by Ah receptor-dependent and -independent pathways. *Toxicol Appl Pharmacol.* 1996; 141:238–247. [PubMed: 8917696]
17. Jeuken A, Keser BJ, Khan E, Brouwer A, Koeman J, Denison MS. Activation of the Ah receptor by extracts of dietary herbal supplements, vegetables, and fruits. *J Agric Food Chem.* 2003; 51:5478–5487. [PubMed: 12926901]
18. Werlinder V, Backlund M, Zhukov A, Ingelman-Sundberg M. Transcriptional and post-translational regulation of CYP1A1 by primaquine. *J Pharmacol Exp Ther.* 2001; 297:206–214. [PubMed: 11259546]
19. Quattrochi LC, Tukey RH. Nuclear uptake of the Ah (dioxin) receptor in response to omeprazole: transcriptional activation of the human CYP1A1 gene. *Mol Pharmacol.* 1993; 43:504–508. [PubMed: 8386305]
20. Arnold LW, LoCascio NJ, Lutz PM, Pennell CA, Klapper D, Haughton G. Antigen-induced lymphomagenesis: identification of a murine B cell lymphoma with known antigen specificity. *J Immunol.* 1983; 131:2064–2068. [PubMed: 6194226]
21. Shi X, Eckhardt LA. Deletional analyses reveal an essential role for the hs3b/hs4 IgH 3' enhancer pair in an Ig-secreting but not an earlier-stage B cell line. *Int Immunol.* 2001; 13:1003–1012. [PubMed: 11470770]
22. Cullen BR. Enhancing and confirming the specificity of RNAi experiments. *Nat Methods.* 2006; 3:677–681. [PubMed: 16929311]
23. Buehler E, Chen YC, Martin S. C911: A bench-level control for sequence specific siRNA off-target effects. *PLoS One.* 2012; 7:e51942. [PubMed: 23251657]

24. Romer EJ, Sulentic CEW. Hydrogen peroxide modulates immunoglobulin expression by targeting the 3'Igh regulatory region through an NFkappaB-dependent mechanism. *Free Radic Res.* 2011; 45:796–809. [PubMed: 21599461]
25. Sulentic CEW, 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]
26. Sulentic CEW, Zhang W, Na YJ, Kaminski NE. 2,3,7,8-tetrachlorodibenzo-p-dioxin, an exogenous modulator of the 3'alpha immunoglobulin heavy chain enhancer in the CH12.LX mouse cell line. *J Pharmacol Exp Ther.* 2004; 309:71–78. [PubMed: 14718603]
27. Sulentic CEW, Kang JS, Na YJ, Kaminski NE. Interactions at a dioxin responsive element (DRE) and an overlapping kappaB site within the hs4 domain of the 3'alpha immunoglobulin heavy chain enhancer. *Toxicology.* 2004; 200:235–246. [PubMed: 15212819]
28. Puga A, Barnes SJ, Chang C, Zhu H, Nephew KP, Khan SA, Shertzer HG. Activation of transcription factors activator protein-1 and nuclear factor-kappaB by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biochem Pharmacol.* 2000; 59:997–1005. [PubMed: 10692565]
29. Suh J, Jeon YJ, Kim HM, Kang JS, Kaminski NE, Yang KH. Aryl hydrocarbon receptor-dependent inhibition of AP-1 activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in activated B cells. *Toxicol Appl Pharmacol.* 2002; 181:116–123. [PubMed: 12051995]
30. Schneider D, Manzan MA, Yoo BS, Crawford RB, Kaminski N. Involvement of Blimp-1 and AP-1 dysregulation in the 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated suppression of the IgM response by B cells. *Toxicol Sci.* 2009; 108:377–388. [PubMed: 19237549]
31. Tian Y. Ah receptor and NF-kappaB interplay on the stage of epigenome. *Biochem Pharmacol.* 2009; 77:670–680. [PubMed: 19014911]
32. Kel A, Reymann S, Matys V, Nettesheim P, Wingender E, Borlak J. A novel computational approach for the prediction of networked transcription factors of aryl hydrocarbon-receptor-regulated genes. *Mol Pharmacol.* 2004; 66:1557–1572. [PubMed: 15342792]
33. Beischlag TV, Luis Morales J, Hollingshead BD, Perdew GH. The aryl hydrocarbon receptor complex and the control of gene expression. *Crit Rev Eukaryot Gene Expr.* 2008; 18:207–250. [PubMed: 18540824]
34. Kim SH, Henry EC, Kim DK, Kim YH, Shin KJ, Han MS, Lee TG, Kang JK, Gasiewicz TA, Ryu SH, Suh PG. Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. *Mol Pharmacol.* 2006; 69:1871–1878. [PubMed: 16540597]
35. Zhao B, Degroot DE, Hayashi A, He G, Denison MS. CH223191 is a ligand-selective antagonist of the Ah (Dioxin) receptor. *Toxicol Sci.* 2010; 117:393–403. [PubMed: 20634293]
36. Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol.* 2003; 43:309–334. [PubMed: 12540743]
37. Stejskalova L, Dvorak Z, Pavek P. Endogenous and exogenous ligands of aryl hydrocarbon receptor: current state of art. *Curr Drug Metab.* 2011; 12:198–212. [PubMed: 21395538]
38. Wood SC, Jeong HG, Morris DL, Holsapple MP. Direct effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on human tonsillar lymphocytes. *Toxicology.* 1993; 81:131–143. [PubMed: 8378939]
39. Kimata H. 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively enhances spontaneous IgE production in B cells from atopic patients. *Int J Hyg Environ Health.* 2003; 206:601–604. [PubMed: 14626908]
40. 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]
41. Ju Z, Volpi SA, Hassan R, Martinez N, Giannini SL, Gold T, Birshtein BK. Evidence for physical interaction between the immunoglobulin heavy chain variable region and the 3' regulatory region. *J Biol Chem.* 2007; 282:35169–35178. [PubMed: 17921139]
42. Birshtein BK. The role of CTCF binding sites in the 3' immunoglobulin heavy chain regulatory region. *Front Genet.* 2012; 3:251. [PubMed: 23162572]

43. Michaelson JS, Singh M, Snapper CM, Sha WC, Baltimore D, Birshtein BK. Regulation of 3' IgH enhancers by a common set of factors, including kappa B-binding proteins. *J Immunol.* 1996; 156:2828–2839. [PubMed: 8609402]
44. Mills FC, Harindranath N, Mitchell M, Max EE. Enhancer complexes located downstream of both human immunoglobulin Calpha genes. *J Exp Med.* 1997; 186:845–858. [PubMed: 9294139]
45. Sepulveda MA, Garrett FE, Price-Whelan A, Birshtein BK. Comparative analysis of human and mouse 3' Igh regulatory regions identifies distinctive structural features. *Mol Immunol.* 2005; 42:605–615. [PubMed: 15607820]
46. Schneider D, Manzan MA, Crawford RB, Chen W, Kaminski NE. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated impairment of B cell differentiation involves dysregulation of paired box 5 (Pax5) isoform, Pax5a. *J Pharmacol Exp Ther.* 2008; 326:463–474. [PubMed: 18483191]
47. Laurencikiene J, Deveikaite V, Severinson E. HS1,2 enhancer regulation of germline epsilon and gamma2b promoters in murine B lymphocytes: evidence for specific promoter-enhancer interactions. *J Immunol.* 2001; 167:3257–3265. [PubMed: 11544313]
48. Chen C, Birshtein BK. Virtually identical enhancers containing a segment of homology to murine 3'IgH-E(hs1,2) lie downstream of human Ig C alpha 1 and C alpha 2 genes. *J Immunol.* 1997; 159:1310–1318. [PubMed: 9233627]
49. Pinaud E, Aupetit C, Chauveau C, Cogne M. Identification of a homolog of the C alpha 3'/hs3 enhancer and of an allelic variant of the 3'IgH/hs1,2 enhancer downstream of the human immunoglobulin alpha 1 gene. *Eur J Immunol.* 1997; 27:2981–2985. [PubMed: 9394827]
50. Giambra V, Fruscalzo A, Giufre M, Martinez-Labarga C, Favaro M, Rocchi M, Frezza D. Evolution of human IgH3'EC duplicated structures: both enhancers HS1,2 are polymorphic with variation of transcription factor's consensus sites. *Gene.* 2005; 346:105–114. [PubMed: 15716094]
51. Frezza D, Tolusso B, Giambra V, Gremese E, Marchini M, Nowik M, Serone E, D'Addabbo P, Mattioli C, Canestri S, Petricca L, D'Antona G, Birshtein BK, Scorza R, Ferraccioli G. Polymorphisms of the IgH enhancer HS1.2 and risk of systemic lupus erythematosus. *Ann Rheum Dis.* 2012; 71:1309–1315. [PubMed: 22294636]
52. Connor KT, Aylward LL. Human response to dioxin: aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. *J Toxicol Environ Health B Crit Rev.* 2006; 9:147–171. [PubMed: 16613807]
53. Harper PA, Wong J, Lam MS, Okey AB. Polymorphisms in the human AH receptor. *Chem Biol Interact.* 2002; 141:161–187. [PubMed: 12213390]

Highlights

- The aryl hydrocarbon receptor (AhR) was inactivated or knocked down by shRNA.
- A regulatory element within the *Igh* gene (3'*IghRR*) is a target of the AhR.
- The AhR is a dominant, negative regulator of the 3'*IghRR*.
- AhR activation inhibits 3'*IghRR* activity and Ig expression.
- AhR disruption reversed inhibition of the 3'*IghRR* and Ig by an AhR ligand.

FIGURE 1.

AhR knockdown and a competitive AhR antagonist decrease TCDD-induced Cyp1A1 expression confirming disruption of the AhR signaling pathway. “Wild Type” (WT) refers to a variant of the CH12.LX murine B-cell line that endogenously expresses IgA and has a stably integrated 3′*Igh*RR-regulated γ 2b transgene (CH12. γ 2b-3′*Igh*RR cells). “shAhR” denotes CH12. γ 2b-3′*Igh*RR cells that stably express shRNA to the AhR via lentiviral-mediated delivery of one of two different shAhR constructs (denoted shAhR11 or shAhR12). A) Whole cell lysates were collected from WT and shAhR cells and 20 μ g of protein was subjected to western blot analysis and probed for the AhR or the loading control β -actin. B) Cells were either treated with 10 nM TCDD or the vehicle control, 0.01% DMSO (not shown), for 8 hours. For the AhR antagonist treatment (first data set), WT cells were either pretreated with 30 μ M of CH-223191 (denoted “AhRA”) or the vehicle control for CH-223191 (0.1% DMSO) for one hour prior to the 8 hr treatment with 10 nM TCDD; the final DMSO concentration was 0.11%. Total RNA was extracted, one μ g was reverse transcribed to cDNA, and 5 ng of cDNA was used to amplify Cyp1A1 and β -actin via SYBR Green quantitative real-time PCR. The Ct method was used to determine the Cyp1A1 levels as a fold-difference or relative quantification (RQ) compared to the highest Cyp1A1-inducing treatment group (WT cells treated with 10 nM TCDD). NA and DMSO treatments caused no appreciable expression of Cyp1A1 (data not shown). Results are representative of at least two separate experiments ($n=3$ for each treatment group). Statistical significance was determined by a one-tailed t-test. “***” and “**” denote significance from the DMSO control at $p<0.001$ and $p<0.01$, respectively.

FIGURE 2.

AhR antagonism reverses TCDD-induced inhibition of the 3'/*Igh*RR-regulated $\gamma 2b$ transgene and endogenous IgA protein expression. CH12. $\gamma 2b$ -3'/*Igh*RR cells were pretreated for 1 h with 30 μ M CH-223191 (denoted "AhRA") or the vehicle control (0.1% DMSO) followed by treatment for 48 h with 10 nM TCDD or the vehicle control (0.01% DMSO) in the presence of LPS (1 μ g/ml) stimulation. A) $\gamma 2b$ and IgA expression (mean $\pm$ S.E., n=4) normalized to 2 μ g total protein was determined by ELISA. B) $\gamma 2b$ and IgA expression transformed to percent effect with the DMSO control set to 100%. The final DMSO vehicle concentration was 0.11%. "C" denotes the LPS alone control and "NA" denotes the naïve control. Significance was determined by a 1-way ANOVA followed by a Dunnett's post-hoc test. "***" and "*" denote significance from the DMSO control at $p < 0.01$ and $p < 0.05$, respectively. "+++" and "++" denote significance of the NA control from the LPS control at $p < 0.001$ and $p < 0.01$, respectively.

FIGURE 3.

AhR knockdown by shRNA reverses TCDD-induced inhibition of the 3'*Igh*RR-regulated $\gamma 2b$ transgene and endogenous IgA protein expression. "Wild Type" (WT) refers to a variant of the CH12.LX murine B-cell line that endogenously expresses IgA and has a stably integrated 3'*Igh*RR-regulated $\gamma 2b$ transgene (CH12. $\gamma 2b$ -3'*Igh*RR cells). "shAhR" denotes CH12. $\gamma 2b$ -3'*Igh*RR cells that stably express shRNA to the AhR via lentiviral-mediated delivery of one of two different shAhR constructs (denoted shAhR11 or shAhR12). Wild Type and shAhR cells were treated with either the vehicle control (0.01% DMSO) or 10 nM TCDD in the presence of LPS (1 μ g/ml) stimulation for 48 hours. $\gamma 2b$ (A) and IgA (B) expression as determined by ELISA in 2 μ g total protein was transformed to percent effect (mean $\pm$ S.E., n=4) with the DMSO control set to 100%. "C" denotes the LPS alone control and "NA" denotes the naïve control. Significance was determined by a 1-way ANOVA followed by a Dunnett's post-hoc test. "*" denotes significance from the DMSO control at $p < 0.05$. "+++" and "++" denote significance of the NA control from the LPS control at $p < 0.001$ and $p < 0.01$, respectively.