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

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), an intronic enhancer (E_μ), and the 3′*Igh* regulatory region (3′*Igh*RR). Primarily activated in terminally differentiated antibody-secreting cells, the 3′*Igh*RR provides for high *Igh* expression as well as class-switch recombination [1]. In humans, the 3′*IGH*RR has been associated with several autoimmune diseases and certain B-cell lymphomas [2–7]. Additionally, we have previously identified the mouse 3′*Igh*RR as a sensitive target of exogenous compounds including dioxins such as 2,3,7,8-tetra chlorodibenzo-ρ-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′*Igh*RR activity by AhR ligands – as well as non-AhR ligands – mirrored the effects on Ig protein levels [14]. Therefore, exposure to

Abbreviations: AhR, aryl hydrocarbon receptor; AhRA, AhR antagonist (CH-223191); ARNT, AhR nuclear translocator; bHLH, basic-helix-loop-helix; CH12.γ2b-3′*Igh*RR, CH12.LX cells stably expressing a 3′*Igh*RR-regulated γ2b transgene; C, LPS alone control; Cyp1A1, cytochrome P4501A1; DMSO, dimethyl sulfoxide; DRE, dioxin response element; E_μ, intronic enhancer; FP, forward primer; IgH, Ig heavy chain; 3′*Igh*RR, mouse 3′*Igh* regulatory region; 3′*IgH*RR, 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.

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environmental triggers may functionally alter 3′*Igh*RR activity leading to altered Ig levels and humoral immunity.

The mouse 3′*Igh*RR 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′*Igh*RR 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′*Igh*RR (reviewed in [1]), the objective of the current study was to determine if the AhR is a significant biological regulator of the 3′*Igh*RR. 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′*Igh*RR-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′*Igh*RR-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′*Igh*RR that at least partially mediates TCDD-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^{a}H-4^{b}p/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 h [14].

Cells were grown in a 37 °C incubator with 5% CO_2 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′-AATTTGCTCATGTTTCAGCGC-3′, corresponding to nucleotide positions 1861–1881 and shAhR12, 5′-TAATAAC ATCTTGCGGGAAGG-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.γ2b-3′*Igh*RR cells (5×10^3 cells/ml) were resuspended in media containing 16 μg/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 μg/ml final concentration of polybrene). Culture plates were immediately centrifuged for 30 min at 1100×*g* before supernatants were discarded and replaced with 200 μL fresh media. After 24 h, cell supernatant was replaced with puromycin (Invivogen, San Diego, CA)-selective media (1 μg/ml). Puromycin-selective media was replaced every 72 h 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′*Igh*RR activation or Ig expression in our shAhR cells following LPS stimulation, supporting 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