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Diverse chemicals including aryl hydrocarbon receptor ligands modulate transcriptional activity of the 3′immunoglobulin heavy chain regulatory region

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ABSTRACT

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a known disruptor of B-cell differentiation and a ligand for the aryl hydrocarbon receptor (AhR), induces binding of the AhR to dioxin responsive elements (DRE) in sensitive genes. The Ig heavy chain (IgH) gene is a sensitive target of TCDD and may be transcriptionally inhibited by TCDD through inhibition of the 3′IgH transcriptional regulatory region (3′IgHRR). While the 3′IgHRR contains binding sites for several transcription factors, two DRE motifs were also identified which may be responsible for TCDD-induced inhibition of 3′IgHRR activation and may implicate the AhR as an important regulator of IgH expression. The objectives of the present study were to determine if 3′IgHRR modulation is limited to TCDD or if structurally diverse chemicals (AhR ligands and non-AhR ligands) from environmental, industrial, dietary or pharmaceutical origin are also capable of modulating the 3′IgHRR and to verify a correlation between effects on a stable 3′IgHRR reporter and the endogenous IgH protein. Utilizing a CH12.LX mouse B-cell line that stably expresses a 3′IgHRR-regulated transgene, we identified an inhibition of both 3′IgHRR activation and IgH protein expression by the non-dioxin AhR activators indolo(3,2-b)carbazole, primaquine, carbaryl, and omeprazole which followed a rank order potency for AhR activation supporting a role of the AhR in the transcriptional regulation of the 3′IgHRR and IgH expression. However, modulation of the 3′IgHRR and IgH expression was not limited to AhR activators or to suppressive effects. Hydrogen peroxide and terbutaline had an activating effect and benzyl isothiocyanate was inhibitory. These chemicals are not known to influence the AhR signaling pathway but have been previously shown to modulate humoral immunity and/or transcription factors that regulate the 3′IgHRR. Taken together these results implicate the 3′IgHRR as a sensitive immunological target and are the first to identify altered 3′IgHRR activation by a diverse range of chemicals.

1. Introduction

2,3,7,8-Tetracholordibenzo-*p*-dioxin (TCDD) is a potent and persistent environmental toxicant, which produces a variety of biological effects in animal and cellular models (Birnbaum and Tuomisto, 2000). Of these effects, immune suppression including inhibition of B-lymphocyte differentiation into antibody-secreting cells is one of the most sensitive consequences of TCDD exposure (Kerkvliet, 2002). Inhibition of Ig expression and secretion in B lymphocytes has been well documented with several studies supporting the involvement of the aryl hydrocarbon receptor (AhR) signaling pathway; however, the specific mechanism remains unclear (Holsapple et al., 1991; Sulentic et al., 1998, 2000; Vorderstrasse et al., 2001).

The AhR and its dimerization partner AhR nuclear translocator (ARNT) are believed to regulate transcription by binding dioxin responsive elements (DREs) in regulatory regions of dioxin-sensitive genes (for review, see Okey, 2007). The CH12.LX B-cell line has provided a useful model in studying the effects of TCDD on Ig expression in B cells and has lead to the identification of a novel transcriptional target of TCDD: the Ig heavy chain (IgH) locus (Sulentic et al., 2000, 2004b). Regulation of the murine IgH locus is governed through a complex interaction of several regulatory elements. One such element, the 3′IgH regulatory region

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AhR, aryl hydrocarbon receptor; DRE, dioxin responsive element; IgH, immunoglobulin heavy chain; 3′IgHRR, 3′IgH regulatory region; ARNT, AhR nuclear translocator; HAHs, halogenated aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; DMSO, dimethyl sulfoxide; ICZ, indolo(3,2,b)carbazole; BITC, benzyl isothiocyanate; PBS, phosphate buffered saline; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; V_H, variable heavy chain; IC_{50}, 50% inhibitory concentration; NF-κB, nuclear factor-κB; Oct, octamer-binding factor; OCA-B, Oct-1-associated coactivator; AP-1, activator protein 1; NF-1, neurofibromatosis type 1.

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(3′IgHRR), appears to mediate processes late in B-cell differentiation such as up-regulation of IgH expression as well as class switch recombination (Manis et al., 1998; Pinaud et al., 2001). The 3′IgHRR is a large, approximately 40 kb region that lies downstream of the IgH constant regions. The 3′IgHRR is most often associated with four enhancer domains (hs3A; hs1,2; hs3B; and hs4) which contain DNA binding sites for several transcription factors that appear to be important regulators of enhancer activity (reviewed by Khamlichi et al., 2000). The AhR may also contribute to the regulation of the 3′IgHRR since at least two functional DRE sites (i.e., TCDD-induced AhR and ARNT binding) have been identified within the hs1,2 and hs4 enhancers (Sulentic et al., 2000). Correspondingly, in the CH12.LX cell line TCDD profoundly inhibited LPS activation of a luciferase reporter construct regulated by the 3′IgHRR (Sulentic et al., 2004b), correlating with the significant inhibition of LPS-induced IgH gene expression and antibody secretion by TCDD and other polychlorinated dibenzo-*p*-dioxin congeners that followed a structure activity relationship for AhR-mediated effects (Sulentic et al., 1998, 2000). Interestingly, the incidence and/or severity of the human diseases, IgA nephropathy and Celiac disease, have been associated with altered regulation of the 3′IgHRR (Aupetit et al., 2000; Frezza et al., 2004). Therefore, chemical-induced modulation of the 3′IgHRR likely has significant implications regarding the potential for altered Ig regulation and impaired immunity.

The most widely studied exogenous AhR ligands are the highly persistent halogenated aromatic hydrocarbons (HAHs), of which TCDD is considered the prototype, and the less persistent polycyclic aromatic hydrocarbons (PAHs). However, it is becoming increasingly evident that exogenous activators of the AhR signaling pathway are not only limited to HAHs or PAHs but also comprise a diverse array of chemicals including those of dietary or therapeutic origin; and studies have also identified indoles, tetrapyroles, and arachidonic acid metabolites as potential endogenous, albeit low affinity, ligands for the AhR (reviewed in Denison and Nagy, 2003; Nguyen and Bradfield, 2008). Therefore an objective of the present study was to determine if the suppression of 3′IgHRR transcriptional activity and Ig expression was dioxin-specific or also sensitive to non-dioxin AhR ligands. However, the 3′IgHRR is clearly regulated by many transcription factors and signaling pathways and consequently may be a sensitive toxicological and clinical target of a broader range of chemicals. Indeed, an additional objective of this study was to validate the utility of our 3′IgHRR model in identifying chemicals that can alter 3′IgHRR activity. Therefore, we expanded our evaluation to chemicals not known to modulate the AhR signaling pathway but shown to modulate the antibody response or signaling pathways involved in 3′IgHRR activity.

Using the well-characterized CH12.LX cell line and both transiently and stably expressed 3′IgHRR reporters, we found that modulation of 3′IgHRR activity was not limited to the AhR ligand TCDD but was sensitive to diverse AhR activators as well as chemicals that influence non-AhR signaling pathways implicated in 3′IgHRR regulation. Additionally, effects on 3′IgHRR activity correlated well with effects on endogenous IgH protein expression. Collectively, these results support the hypothesis that the 3′IgHRR is a sensitive immunological target of a diverse range of chemicals and that altered 3′IgHRR activity will alter IgH expression and antibody-mediated immune function. Furthermore, these results not only support a role of the AhR in 3′IgHRR transcriptional activity but also underscore the complexity of 3′IgHRR regulation and the diversity of chemicals that may alter 3′IgHRR activity.

2. Materials and methods

2.1. Chemicals and reagents

TCDD, 1-monochlorodibenzo-*p*-dioxin (MCDD), and 2,2′,3,5′,6-pentachlorobiphenyl (PCB95) in 100% dimethyl sulfoxide (DMSO) were purchased from AccuStandard Inc. (New Haven, CT). Hydrogen peroxide solution (30 wt.% in water) and the neat form of carbaryl, primaquine, omeprazole, terbutaline, and carbachol were purchased from Sigma–Aldrich (Milwaukee, WI). The certificates of product analysis stated the purity of TCDD, MCDD, PCB95, primaquine, carbaryl, omeprazole, terbutaline, and carbachol to be 99.1%, 100%, 100%, 98%, 98.9%, 100%, 99% and 99%, respectively. Indolo(3,2,b)carbazole (ICZ) was generously provided by Dr. Leonard F. Bjeldanes (University of California, Berkely, CA). ICZ, carbaryl, primaquine, and omeprazole were dissolved in DMSO. Terbutaline and carbachol were dissolved in water. Benzyl isothiocyanate (BITC) dissolved in a 50% DMSO phosphate buffered saline (PBS) solution was generously provided by Dr. Sanjay Srivastava (Texas Tech University) and Dr. Thomas Brown (Wright State University). See Table 1 for a list of the chemicals tested and a summary of their purity, vehicle, final vehicle concentration, and range of concentrations tested. Lipopolysaccharide (LPS, *Escherichia coli*) and DMSO were purchased from Sigma–Aldrich.

2.2. Cell lines

The CH12.LX B-cell line derived from the murine CH12 B-cell lymphoma (Arnold et al., 1983), which arose in B10.H-2^{a}H-4^{b}p/Wts mice (B10.A × B10.129), has been previously characterized by Bishop and Haughton (1986) and was a generous gift from Dr. Geoffrey Haughton (University of North Carolina, Chapel Hill, NC). Since its initial characterization, the CH12.LX cell line has been extensively utilized to study a variety of cellular processes specific to B cells and has provided a useful model in studying the effects of TCDD on B-cell differentiation. Employing the γ2b mini-locus model which was developed and generously provided by Dr. Laurel Eckhardt (Hunter College, The University of New York City, NY) (Shi and Eckhardt, 2001), we generated the CH12.γ2b-3′IgH cell line. The CH12.γ2b-3′IgH cell line is a subclone isolated from CH12.LX cells that were under antibiotic selection to induce the stable expression of a transgene (γ2b Ig heavy chain gene) regulated by the 3′IgHRR. Inducible expression of the γ2b transgene in the CH12.γ2b-3′IgH was confirmed by real-time RT-PCR and enzyme-linked immunosorbent assay (ELISA) analysis (data not shown and Fig. 2). Analysis of the CH12.γ2b-3′IgH cells by flow cytometry and ELISA identified them as IgA expressing B cells and genomic analysis identified insertion of one copy of the γ2b transgene (Figs. 5 and 7 and data not shown). The CH12.γ2b-3′IgH

Table 1
Test chemicals. Non-dioxin AhR ligands include ICZ, primaquine, carbaryl, and omeprazole. Negative controls for AhR binding include 1-monochlorodibenzo-*p*-dioxin and the non-coplanar PCB95 both of which have no affinity for the AhR. Chemicals not known to modulate the AhR signaling pathway but shown to modulate the antibody response or signaling pathways involved in 3′IgHRR activity include hydrogen peroxide, terbutaline, carbachol, and BITC. DMSO vehicle concentrations varied according to the solubility and effective concentration range of the chemical.

Chemical	Purity	Vehicle	Final vehicle concentration	Concentration range tested
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	99.1%	100% DMSO	0.01% or 0.019% DMSO	0.01–30 nM
Indolo(3,2,b)carbazole ICZ	ND	100% DMSO	0.01% DMSO	0.5–500 nM
Primaquine (PMQ)	98%	100% DMSO	0.01% DMSO	6.25–100 μM
Carbaryl (CBRL)	98.9%	100% DMSO	0.1% DMSO	12.5–100 μM
Omeprazole (OME)	100%	100% DMSO	0.1% DMSO	12.5–62.5 μM
1-Monochlorodibenzo-*p*-dioxin (MCDD)	100%	100% DMSO	0.01% or 0.1%	30 and 300 nM
Polychlorinated biphenyl 95 (PCB95)	100%	100% DMSO	0.01% or 0.1%	30 and 300 nM
Hydrogen Peroxide (H_2O_2)	ND	Water	NA	20–50 μM
Terbutaline (TERB)	99%	Water	NA	0.1–100 μM
Carbachol (CBOL)	99%	Water	NA	0.01–100 μM
Benzyl isothiocyanate (BITC)	ND	50% DMSO 1× PBS	0.05% DMSO	0.5–5.0 μM

ND, not determined; NA, not applicable.