Role of Aryl Hydrocarbon Receptor Polymorphisms on TCDD-mediated Suppression of Human B cell Function

Norbert E. Kaminski, Ph.D.

Center for Integrative Toxicology
Department of Pharmacology & Toxicology

Michigan State University
East Lansing, MI
Seminar Objectives

- Background on NIEHS P42 Superfund Research Program Center Grants and specifically MSU P42 Center.
- Rationale for focusing on the B cell as a sensitive target for TCDD-mediated immunotoxicity.
- Discuss human B cell models both primary cell and cell line-based for mechanistic studies.
- Discuss the role of AHR polymorphisms in B cell responses to TCDD.
- Discuss modulation of the IgM response and Cyp1a1 induction as an endpoint to assess TCDD bioavailability in the context of interactions with naturally occurring environmental matrices and remediation materials.

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Superfund Basic Research Program
P42 Center Grants

- Multidisciplinary research programs focused on a unifying central theme concerning “Superfund designated environmental contaminants”

- Comprised of both biomedical and remediation sciences research projects as well as supporting “cores” for research, training, community engagement and research translation.

- Research is both mechanistic and applied with an emphasis on problem solving in the context of minimizing exposure and facilitating environmental remediation.

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Environmental, Microbial, and Mammalian Biomolecular Responses to AHR Ligands

Administered and coordinated by MSU

4 Biomedical Projects
2 Remediation Engineering Projects
6 Core Facilities
Rutgers
Purdue
Texas A&M
Emory
US EPA
25 Investigators

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2,3,7,8-tetrachlorodibenzo-p-dioxin
Profile of Biological Activity by TCDD

- enzyme induction
- hepatomegaly
- lymphoid involution primarily thymus
- immune modulation (i.e. mostly suppression)
- chloracne and epithelial hyperplasia
- teratogenesis (example: cleft palate)
- cancer (tumor promoter)
- wasting syndrome
- death

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The AHR Signaling Cascade

PCDD, PCDF, ARA-9

AhR

Downstream Targets

PCBs, PBBs

Cytosol

RNA

Nucleus

Gene regulation

AhR

ARNT

DNA

DRE

TNGCGTG

AhR

ARNT

DRE
Why are we focused on the B cell?

- The antibody response has historically been one of the most sensitive indicators of TCDD immune toxicity.

- Spleen cell fractionation-reconstitution experiments show that the B cell is the primary cellular target in suppression of antibody responses.

- Direct effects of TCDD on B cell function have been shown in B cell lines and isolated 1st B cells.
Major Issues

Are human B cells sensitive to suppression of effector function by TCDD and DLC?

What strategies can be employed to make comparisons across species?
Overall Strategy for Species Comparisons

- When possible, employ primary B cells to insure that effects observed are not an artifact of the biological model.

- If possible, use the same cell activation system across species.
CD40-CD40L Interactions Provide a Crucial Signal for B cell Differentiation

Antibody secreting cells (plasma cells)

Memory cell

Antigen

B

CD154 (CD40L)

CD40

T_H2

cytokines

IL-2, IL6, IL-10

(Modified from Janeway’s Immunobiology)
Isolation of Primary B cells

Leukocyte suspension is prepared

Biotin-conjugated antibody ‘cocktail’ against non-target cells

B cells approximately >96% pure

Superfund Research Program Annual Meeting Nov. 3-4, 2009
CD40L-induced IgM Response Model using HPB B cells

Phase I

Cytokines

B cell

CD40

CD40L-L cell (Irradiated)

Day 0

Phase II

Cytokines

B cell

New culture plate without CD40L-L cell

Day 4

Phase III

ELISA

IgM

IgM secreting cell

AFC assay

ELISPOT

Day 7

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Enumeration of IgM Secreting HPB B Cells by ELISPOT

Non-activated HPB B cell

CD40L-activated HPB B cell

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TCDD Effect on CD40-induced IgM Response in Purified Mouse B Cells

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Suppression of CD40L-Induced IgM Response by TCDD in Human Peripheral Blood B cells

* Approximately 1 in 6 donor is unresponsive to TCDD-mediated suppression of IgM response.
CD40L-induced IgM Response in Human B Cells
“Nonresponsive” Donors

Haitian Lu, 2010
Variability in Sensitivity to TCDD Among Mouse Strains

- Sensitivity to TCDD-induced toxic effects is 10 fold higher in C57BL/6 as compared to DBA/2 mice.

- DBA mice express polymorphic form of the AHR, which contains an alanine to valine (A375V) amino acid substitution in the ligand-binding domain.
The absence of suppression by TCDD of primary humoral immune responses for 1 in 6 donors is due to polymorphisms within aryl hydrocarbon receptor.
Sites of Known AHR Polymorphisms

-459 G>A  
132 T>C  

Generate SKW cell lines expressing:
1) 517 C>T  
2) 554 G>A  
3) 570 G>A  
4) 570+517  
5) 570+554  
6) 570+554+517

A.B.Okey. Chemico-Biological Interactions. 2002

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## NHLBI Exome Sequencing Project (ESP) Exome Variant Server

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**European American (EA)**
**African American (AA)**

MAF - minor allele frequency

http://evs.gs.washington.edu/EVS/

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Human SKW 6.4 Cells as a Model of TCDD-mediated B cell Toxicity

SKW 6.4 B cell line is a human EBV-transformed lymphoblastoid cell line, arrested at a late stage of differentiation.

SKW cells produce IgM in response to LPS and/or PWM treatment.

SKW cells do not express the gene that codes for the AHR.
SKW-AHR⁺ Cell Line

- cDNA for the AHR was amplified from the human hepatoma cell line (HepG2).
- The transduction vector was engineered to provide induced expression of the gene of interest in the presence of doxycycline.

The AHR transduction vector was designed to result in the expression of AHR-GFP fusion protein.
AhR mRNA Expression Levels

![Bar graph showing AhR mRNA expression levels for different cell types:]

- ND
- SKW 6.4
- SKW-AHR+
- HepG2
- Human Primary B Cells

**X-axis:** AHR mRNA (ΔCT)

**Y-axis:** ND

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AHR Western Blot

- SKW6.4
- SKW6.4-AHR⁺
- HepG2
- Human B cells

- AHR-GFP 130 kDa
- AHR 95 kDa
- β-actin 42 kDa

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Kinetics of CYP1B1 Induction in SKW-AHR+ Cells

Cyp1B1 mRNA Levels (Fold Change)

Time (hr)

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LPS-Induced IgM Response in the Presence of TCDD Treatment

SKW

SKW AHR^+
AHR Protein Expression as Determined by Western Blotting

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Cyp1A2 mRNA Levels in the Presence of TCDD Treatment
Cyp1B1 mRNA Levels in the Presence of TCDD Treatment

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Cyp1B1 Luciferase Reporter Activity in the Presence of TCDD Treatment
AHR Protein Expression as Determined by Western Blotting
Effects of TCDD on the LPS-Induced IgM Response in the Presence of TCDD

TCDD (0.3, 1, 3, 10, 30 and 100 nM)
AHR polymorphism 554 decreased TCDD-mediated induction of CYP1A2 and CYP1B1 mRNA levels as well as CYP1B1 reporter activity.

AHR polymorphism 554 did not attenuate TCDD-mediate suppression of the IgM response.

SKW cells stably transduced with AHR containing polymorphisms V570I+P517S+R554K did exhibit modest attenuation of TCDD-mediate suppression of the IgM response.
Conclusions

- Since polymorphism 554 is located in the AHR transactivation domain, one possibility for attenuation of TCDD-mediated CYP induction but not IgM suppression could be due to the involvement of different coactivators and/or corepresensors in the two different biological responses.

- Alternatively unlike TCDD-mediated CYP induction, suppression of the IgM response by TCDD is not due to direct transcriptional regulation by the AHR of genes involved in the B cell differentiation program resulting in IgM production.
P42 Centers are also charged with contributing to development of novel remediation strategies as well as providing new insights in the area of exposure to Superfund designated environmental contaminants.
Tittabawassee River
Investigate the interactions of dioxin-like compounds with naturally occurring environmental matrices and remediation materials.

- Silica
- Clay
- Activated Carbon
Experimental Design

antigen sensitization (sRBC)

1. administration of TCDD adsorbed onto matrices or in corn oil by oral gavage
2. collect tissues (spleen and liver)
3. Quantify anti-sRBC IgM AFC response
4. Cyp1a1 mRNA levels in spleen and liver
Bioavailability of Orally Administered TCDD Adsorbed Silica: Effects on the IgM AFC Response

Toxicology 282:82-87, 2011
Bioavailability of Orally Administered TCDD Adsorbed Silica: Effects on CYP1A1 mRNA Induction

Spleen

Liver

Toxicology 282:82-87, 2011
Bioavailability of Orally Administered TCDD Adsorbed Clays: Effects on the IgM AFC Response

Bioavailability of Orally Administered TCDD Adsorbed Clays: Effects on CYP1A1 mRNA Induction in Liver

Bioavailability of Orally Administered TCDD Adsorbed Activated Carbon: Effects on the IgM AFC Response

![Graph showing antibody response vs. TCDD dose]

- Activated Carbon
- Corn Oil

*Significant differences compared to Vehicle
Bioavailability of Orally Administered TCDD Adsorbed Activated Carbon: Effects on CYP1A1 mRNA Induction in Liver

[Graph showing fold induction of CYP1A1 mRNA with TCDD in CO and TCDD-absorbed Activated Carbon]
Conclusions

- TCDD adsorbed onto silica as well as on either natural K saponite or synthetic saponite clay was bioavailable when administered orally.

- TCDD adsorbed onto activated carbon possessing micropores was not bioavailable when administered orally.
Acknowledgements

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