AhR Ligand-Specific Effects on Dendritic Cell Activation

Kristina Finsaas, Andrea Miller, and David Shepherd
Center for Environmental Health Sciences, Department of Biomedical & Pharmaceutical Sciences, University of Montana, Missoula, MT 59812

Abstract

The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that when activated, results in immunomodulation. Dendritic cells (DCs) are important immune cells involved in innate and adaptive immune responses. Currently, it is not fully understood if different AhR ligands differentially affect DCs. We hypothesized that DC activation will be inhibited following AhR activation in an AhR ligand-specific manner. To test this hypothesis, we evaluated the effects of five disparate AhR ligands: TCDD, Benzo (a)pyrene, FICZ, Indole-3-carbinol and Indirubin on LPS-stimulated DC 2.4 cells. Alterations in DC accessory molecules (CD40, CD80, CD86 and MHC class I) and the production of inflammatory mediators (IL-6, TNF-α and nitric oxide) were assessed. Cell number and viability were assessed following treatment with all five compounds. All AhR ligands decreased the LPS-stimulated production of the pro-inflammatory cytokines, IL-6 and TNF-α, by the cultured DCs while only Indole-3-carbinol and Indirubin inhibited the generation of nitric oxide. As expected, LPS increased the relative expression of MHC class I, CD40, CD80 and CD86 on the DC2.4 cells. Relative to the vehicle-treated controls, TCDD increased the expression of CD40 and CD80; Benzo(a)pyrene decreased CD80; FICZ did not change any of the accessory molecules; Indole-3-carbinol decreased CD80 and CD86; and Indirubin decreased all four surface molecules. Collectively, these results suggest that DCs respond differently to these five distinct AhR ligands, effects that are ultimately expected to alter the generation of immune responses mediated by DCs.

Introduction

Dendritic cells are very important cells in the immune system. They are responsible for the uptake and presentation of antigens from the tissue periphery to T cells, enabling the generation of the adaptive immune response and the elimination of microbial pathogens. But this process can be interrupted by exposure to AhR ligands, changing how the immune system functions. Thus, the AhR plays an integral role in how the adaptive immune system responds to different chemicals in the environment.

The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor found in the cytosol. The AhR is a common pathway that mediates the immunotoxicity of many xenobiotics. However, it is not currently understood how various AhR ligands affect DCs or the immune system.

- 2,3,7,8-Tetrachlorodibenzo-p-dioxin (**TCDD**)
  - Halogenated aromatic hydrocarbon (HAH)
  - Formed by industrial processes
  - Prototypical AhR ligand
- Benzo[a]pyrene (**BaP**)
  - Polycyclic aromatic hydrocarbon (PAH)
  - By-product of automobile exhaust and cigarette smoke
  - Strong AhR ligand
- 6-formylindolo[3,2-b]carbazole (**FICZ**)
  - Natural AhR ligand
  - Tryptophan metabolite
- Indole-3-carbinol (**IC3**)
  - Natural AhR ligand
  - Found in cruciferous vegetables
- Indirubin-3´-oxime (**INDR**)
  - Natural AhR ligand
  - Found in the indigo plant and commonly used in traditional Chinese medicine

This study investigated the effects of TCDD, BaP, FICZ, IC3, and INDR on DCs by evaluating the changes that occur following DC activation by LPS, a constituent of gram-negative bacterial cell walls. This study primarily serves to further establish the effects of AhR activation in DCs and model how immune cells respond to environmental toxic pollutants during bacterial infection.

Role of Dendritic Cells in the Immune System

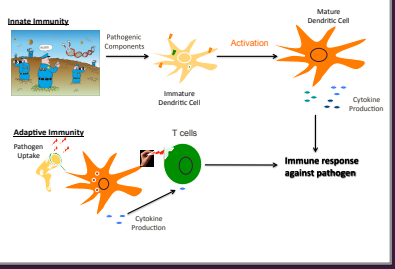


Figure 1: Dendritic cells are integral cellular contributors to innate and adaptive immune responses.

AhR Signaling Pathway

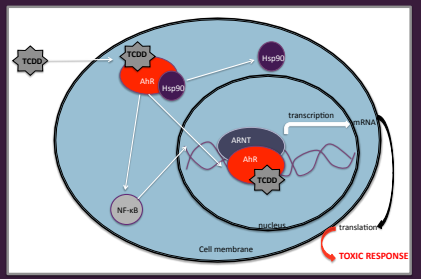


Figure 2: When bound by an AhR ligand, the AhR can be activated by two signaling pathways. In the canonical pathway, AhR dissociates from its chaperone proteins and migrates to the nucleus where it heterodimerizes with the Aryl hydrocarbon receptor nuclear translocator (ARNT) and modulates gene transcription via Dioxin response elements (DREs). In the non-canonical pathway, AhR can alter NF-κB signaling, ultimately affecting transcription.

Materials and Methods

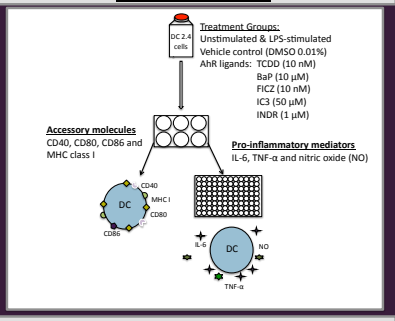


Figure 3: DC2.4 cells were seeded at $0.5x10^6$ cells/mL/well into 6-well plates (n=4). Cells were either treated with Vehicle, TCDD, BaP, FICZ, I3C and INDR, and incubated for 48 hours. After harvest, inflammatory mediator production and cell surface molecule expression were evaluated by ELISA or flow cytometry, respectively.

RESULTS

Dendritic Cell Number and Viability

Cell Number

	Unstimulated						LPS					
	Vehicle	TCDD	BaP	FICZ	IC3	INDR	Vehicle	TCDD	BaP	FICZ	IC3	INDR
Experiment 1	0.4 ± 0.1	1.0 ± 0.5	0.6 ± 0.1	0.5 ± 0.1	ND	ND	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	ND	ND
Experiment 2	5.3 ± 0.6	5.7 ± 0.2	ND	ND	4.0 ± 0.0	5.0 ± 0.3	1.0 ± 0.2	2.4 ± 0.3	ND	ND	**2.4 ± 0.4***	**2.0 ± 0.3***

Viability

	Unstimulated						LPS					
	Vehicle	TCDD	BaP	FICZ	IC3	INDR	Vehicle	TCDD	BaP	FICZ	IC3	INDR
Experiment 1	73 ± 6.9	81 ± 4.0	66 ± 4.0	68 ± 5.2	ND	ND	79 ± 3.1	74 ± 4.0	70 ± 1.4	81 ± 3.7	ND	ND
Experiment 2	93 ± 0.2	92 ± 0.5	ND	ND	90 ± 0.7	91 ± 0.8	75 ± 1.3	88 ± 1.1	ND	ND	82 ± 1.6	84 ± 0.8

Figure 4: Cell number and viability was calculated for each treatment group after a 48hr incubation period. Despite changes in cell number, cell viability stays relatively constant across all treatment groups.

Pro-inflammatory Mediator Production

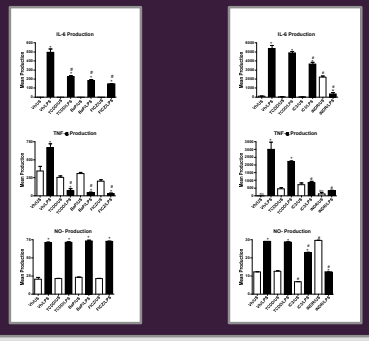


Figure 5: The effects of a panel of AhR ligands on the production of IL-6, TNF-α and nitric oxide (NO) by DC2.4 cells. DCs were unstimulated or stimulated with LPS for 48 hrs, supernatants collected and analyzed by ELISA (IL-6 and TNF-α) or the Greiss reaction (NO).
* significant difference unstimulated vs. LPS
# significant difference treated groups vs. respective control

Costimulatory Molecule Expression

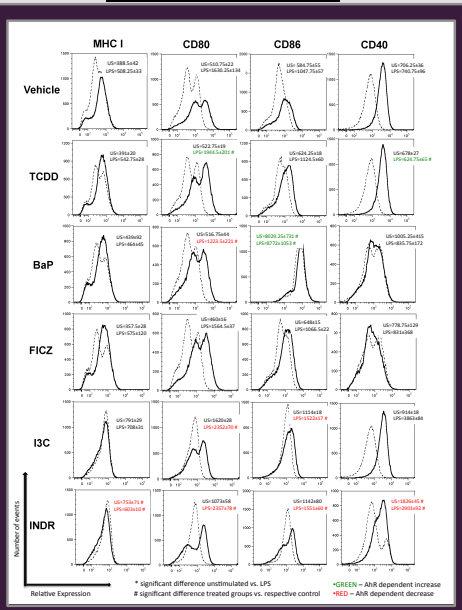


Figure 6: The effects of a panel of AhR ligands on the expression of CD40, CD80, CD86 and MHC I by DC 2.4 cells. DCs were treated with LPS and Vehicle, TCDD, BaP, FICZ, I3C, INDR for 48hrs. Cells were harvested, blocked for non-specific staining, and labeled with optimally titrated monoclonal antibodies specific for the accessory molecules. DC2.4 cells were then analyzed on a BD Biosciences Aria Flow cytometer and representative histograms generated using FlowJo software. Individual histograms are shown with corresponding mean values ± SEM for all treatment groups.

Summary

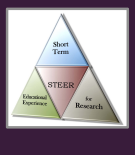
- I3C and INDR increased DC2.4 cell numbers following LPS activation.
- No effects were observed on DC viability following treatment with all five AhR ligands.
- All AhR ligands examined decreased the LPS-stimulated production of the pro-inflammatory cytokines, IL-6 and TNF-α.
- I3C and INDR inhibited the production of nitric oxide.
- TCDD increased expression of CD40 and CD80.
- BaP decreased CD80.
- FICZ did not affect any of the accessory molecules tested.
- I3C decreased CD80 and CD86.
- INDR decreased MHCI, CD40, CD80 and CD86.

Conclusions

- I3C and INDR inhibit DC proliferation.
- At the concentrations tested, all five AhR ligands are not cytotoxic.
- AhR activation in DCs attenuates the production of IL-6 and TNF-α.
- Accessory molecules were most sensitive to INDR>TCDD=I3C>BaP>FICZ.
- DCs are sensitive to AhR activation.

Future Directions

- Examine bone marrow-derived dendritic cells (BMDCs) to establish if primary DCs are affected similar to DC2.4 cells.
- Determine if the AhR ligand-specific effects in DCs are AhR-dependent by using BMDCs from AhR knockout mice.
- Determine if these different ligands mediate their specific effects via the canonical or non-canonical AhR signaling pathways.
- Assess cell cycle changes in the DCs after AhR activation.



Acknowledgements

The project described was supported by Award Number R25ES016247 and R01ES13847 (DMS) from the National Institute Of Environmental Health Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Environmental Health Sciences or the NIH. Thanks to Jenna Benson and Tom Simones for their assistance in data presentation.