Published in final edited form as:

Toxicol Environ Chem. 2012 ; 94(6): 1175–1187. doi:10.1080/02772248.2012.688546.

The Aryl Hydrocarbon Receptor Influences Transplant Outcomes in Response to Environmental Signals

S. Kyle Pauly¹, John H. Fechner¹, Xiaoji Zhang¹, Jose Torrealba², Christopher A. Bradfield^{3,4}, and Joshua D. Mezrich^{1,5}

¹Division of Transplantation, Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792

²Department of Surgical Pathology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792

³McArdle Laboratory for Cancer Research, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792

Abstract

The aryl hydrocarbon receptor (AHR) is a cytosolic transcription factor with numerous endogenous and xenobiotic ligands, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Recent data suggests that TCDD may induce regulatory T cells, while a second AHR ligand, FICZ, promotes Th17 differentiation. The aim was to examine whether injection of recipient mice with either TCDD or FICZ altered skin allograft rejection in a fully mismatched model. TCDD or FICZ was given to recipient C57BL/6 mice intraperitoneally (IP). Twenty-four hr later, donor skin was grafted from BALB/c mice. An additional dose of FICZ was given on day 3. Treatment with TCDD delayed graft rejection for more than 4 weeks while FICZ treatment accelerated rejection by 1 – 2 days. In vivo exposure with TCDD led to a rise in the frequency of FoxP3⁺ CD4⁺ T cells in the spleen, while FICZ increased IL-17 secretion by splenocytes from treated animals. Activation of the AHR receptor by different AHR ligands in vivo resulted in opposing effects on skin graft survival. AHR serves as a sensor to environmental signals, with effects on the acquired immune system that may alter outcomes after organ transplantation. This model will be useful to further delineate direct effects of the environment on the immune system and outcomes of organ transplantation.

INTRODUCTION

The aryl hydrocarbon receptor (AHR) is best known as the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), a potent environmental toxicant. Activation of

⁵Address correspondence to: Joshua Mezrich, M.D., Department of Surgery, University of Wisconsin School of Medicine and Public Health, H4/754 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792-7375. mezrich@surgery.wisc.edu.

S. Kyle Pauly, University of Wisconsin School of Medicine and Public Health, H4/754 Clinical Science Center, 600 Highland Ave., Madison, WI 53792. Phone – (608) 265-6471. Fax – (608) 262-6280. skpauly@wisc.edu

John H. Fechner, University of Wisconsin School of Medicine and Public Health, H4/754 Clinical Science Center, 600 Highland Ave., Madison, WI 53792. Phone – (608) 265-6471. Fax – (608) 262-6280. fechner@surgery.wisc.edu

Xiaoji Zhang, University of Wisconsin School of Medicine and Public Health, H4/754 Clinical Science Center, 600 Highland Ave., Madison, WI 53792. Phone – (608) 265-6471. Fax – (608) 262-6280. zhangxi@surgery.wisc.edu

Jose Torrealba, Department of Surgical Pathology, C7/350-3224, 600 Highland Ave., Madison, WI 53792. Phone – (608) 265-4375. Fax – none. jrtorrea@wisc.edu

Christopher A. Bradfield, 213 A McArdle Laboratory, University of Wisconsin School of Medicine and Public Health, 1400 University Ave., Madison, WI 53706. Phone – (608) 262-2024. Fax – (608) 262-2824. bradfield@oncology.wisc.edu

Joshua D. Mezrich, H4/754 Clinical Science Center, 600 Highland Ave., Madison, WI 53792. Phone – (608) 265-6471. Fax – (608) 262-6280. mezrich@surgery.wisc.edu

⁴C.A.B. has served as a scientific consultant to DOW Chemicals on issues related to dioxin toxicity.

the AHR leads to hepatocellular damage, thymic involution, cancer, and immunosuppression (Schmidt *et al.* 1993). The AHR also has a role in normal organ development, as the AHR null mouse (AHR^{-/-}) has phenotypic abnormalities in hepatic vascular development (Schmidt *et al.* 1996). There are many known ligands of the AHR in addition to TCDD, including 6-formylindolo[3,2-b]carbazole (FICZ). This ligand is formed by exposure of tryptophan (Trp) to UVB light (Fritsche *et al.* 2007, Rannug *et al.* 1995). It was proposed that skin exposure to excessive sunlight leads to tryptophan metabolism to FICZ and other photoproducts, that ultimately activate the AHR in cells present in the dermis (Diani-Moore *et al.* 2006, Jux *et al.* 2011, Nair *et al.* 2009).

Recently, data emerged indicating a strong role for the AHR in T-cell differentiation. Quintana *et al.* (2008) demonstrated that activation of the AHR by TCDD leads to the differentiation of naïve CD4⁺ T cells into FoxP3⁺ regulatory T cells (Tregs). Veldhoen *et al.* (2008) showed that AHR activation via FICZ enhanced the differentiation of naïve CD4⁺ T cells into Th17 cells. Activation of the AHR *in vivo* by TCDD reduced the severity of disease in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis, while treatment with FICZ increased the severity of disease.

Several reports described the capacity of AHR ligands to alter the phenotype of dendritic cells (DC) (Nguyen *et al.* 2010, Quintana *et al.* 2010, Simones and Shepherd 2011). Recent studies suggested that AHR may lead to an up-regulation of indoleamine 2,3-dioxygenase (IDO) (Bankoti *et al.* 2010, Jux *et al.* 2009, Mezrich *et al.* 2010, Vogel *et al.* 2008). IDO is the rate-limiting enzyme controlling degradation of Trp along the kynurenine pathway (Jux *et al.* 2009, Mellor and Munn 2004). IDO is present and active in subsets of DC, and thought to be central to the mechanism responsible for the generation of Tregs during DC-T cell interactions (W. Chen *et al.* 2008, Mellor and Munn 2004). The exact cellular pathway by which IDO leads to Tregs has been debated; both Trp starvation and direct effects of Trp metabolites were proposed (Fallarino *et al.* 2006, Frumento *et al.* 2002, Terness *et al.* 2002).

Based on these effects on immune cells, it was hypothesized that ligands of the AHR might alter graft survival after transplantation. Skin transplantation was utilized as a well-defined model to assess rejection and the effects that AHR ligands exert on transplant survival compared to controls.

Methods and Material

Animals

Six to 12 week old male C57BL/6J (B6; H-2^b) and BALB/c (H-2^d) mice weighing 20–30 grams were obtained from The Jackson Laboratory (Bar Harbor, ME). AHR-null mice on a C57BL/6J background (Schmidt *et al.* 1996) were bred and maintained under specific pathogen-free conditions. Animal experiments were carried out according to institutional guidelines.

Skin Transplantation

Full-thickness skin derived from Balb/c or C57BL/6J donor mice was transplanted on the dorsal area of C57BL/6J recipient. Necrosis of 80% of the transplanted skin surface was considered rejection, and all assessments were performed by blinded observers. In the first experiment, 4 groups of wild-type B6 mice underwent skin transplantation: untreated syngeneic group, untreated allogeneic group, and a syngeneic and allogeneic group treated with a single intraperitoneal (ip) injection of TCDD at 50 µg/kg on post-operative day 1 (POD-1). For the second experiment, there were 4 conditions: allogeneic graft on untreated mice (n=7), allogeneic graft on FICZ-treated mice (Sigma, St. Louis, MO) (ip, 100 µg/kg on POD -1 and 4) (n=12), allogeneic graft on DMSO-treated mice (diluent for FICZ IP, POD -1

and 4) and allogeneic graft on AHR-nulls (n=6). All mice were anesthetized for bandage removal on POD 6. Dosing regimens were based on previous experiments in experimental autoimmune encephalomyelitis (EAE) (Quintana *et al.* 2008, Veldhoen *et al.* 2008), and on knowledge of the rapid metabolism of FICZ and minimal metabolism of TCDD (Johnson *et al.* 1992, Wei *et al.* 2000). Graft survival for untreated mice and mice receiving diluent alone were not significantly different.

Histology

All skin grafts were harvested after euthanizing the recipient mouse when specified. Skin tissue was fixed in 10% formalin, embedded in paraffin, cut into 5 μ m of sections and stained with hematoxylin and eosin.

Flow Cytometry Analysis of Lymphocytes

Spleen, axillary lymph node, thymus and blood were harvested from mice at the time of euthanasia. Lymphocytes were stained with antibodies: CD3 (145-2C11), CD25 (PC61.5), Foxp3 (FJK-16s) (eBioscience, San Diego, CA); Ki67(B56), CD4 (RM4-5), CD8(53-6.7) (BD Biosciences). For intracellular staining of Foxp3 and Ki67, cells were first surface stained and then fixed and permeabilized with the fixation/permeabilization buffer (eBioscience). Acquisition was done on a FACS Caliber or LSR II (BD Biosciences).

Isolation and differentiation of bone marrow-derived DCs

Isolation of murine bone marrow-derived DC (BMDC) was performed as previously described (Mezrich *et al.* 2010). Greater than 80% of the cell population stained positive for CD11c by flow cytometry.

Real-time quantitative PCR

Total RNA was extracted using RNeasy Mini Kits and RNase-Free DNase Sets (Qiagen, Valencia, CA). A total of 500 ng total RNA in each group was used for RT reaction (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA; or High-capacity cDNA Reverse Transcription Kits, Applied Biosystems, Foster City, CA). The relative quantitation PCR for IDO1 (Mm00492586-m1) and GAPDH (4352339-E0806018), and performed in the Applied Biosystems 7900HT Fast Real-Time PCR System, and TaqMan Universal PCR Master Mix used as a reaction reagent. The relative quantitation PCR for Foxp3, Cyp1a1, Cyp1b1, TGF- β 1, and GAPDH were processed by the Bio-Rad iCycler and iQ SYBR Green Supermix was used as the reaction reagent.

Isolation of naive CD4⁺ T cells and T cell differentiation

Naive CD4⁺ T cells were isolated using the CD4⁺ CD62L Isolation Kit (Miltenyi Biotec, Auburn, CA) and an autoMACS. For quantitative PCR (qPCR) analysis, $2-5 \times 10^5$ cells were cultured in each well of a 96- well round-bottom plate coated with 0.5 μ g/ml anti-CD3 and anti-CD28 overnight and then washed with PBS twice before seeding the cells. The naive T cells were maintained in F10 media and were treated with 10 nM TCDD, 100 nM FICZ or 2–10 ng/ml TGF- β (as specified). After 5 days, the cultured cells were harvested for qPCR assay.

pDC/T cell coculture

Naive CD4⁺CD25⁻ T cells were isolated from WT and AHR-null mice and cocultured with BALB/c pDC isolated using the Miltenyi Mouse pDC Isolation Kit (Miltenyi Biotec) at a ratio of 20:1 or 10:1 as previously described (Mezrich *et al.* 2010). TCDD (10nM) OR FICZ (100nM) were added at the start of culture. On day 5, cells were harvested and subjected to qPCR analysis.

Statistics

Statistical differences of skin graft survival data was determined using by the Kaplan-Meier analysis. Two-way ANOVA with Bonferroni post-ANOVA testing was used for all other statistical analysis. Statistical significance was defined as a p value of < 0.05. Data are showed as Mean $\pm$ Standard Deviation. All statistical analysis was performed using Prism 5 software (GraphPad, San Diego, CA).

RESULTS

A single dose of TCDD delays rejection of fully mismatched skin grafts

Utilizing the Balb/c (H-2^d) to C57BL/6 (H-2^b) model of skin allograft transplantation, six B6 recipient mice were given a single dose of TCDD one day prior to transplant and were then euthanized on post-operative days (POD) 10 or 16, or at the time of rejection (Figure 1A). Compared to untreated (median GST=10 days), no graft loss from rejection was seen in the TCDD-treated group in the first 30 days post-transplant. This difference was found to be statistically significant (Figure 1B). Gross examination of skin grafts from TCDD-treated mice revealed viable skin with hair growth through POD 29 (Supplemental Figure 1). Histological examination at POD 10 and 16 revealed evidence of a cellular infiltrate indicating mild cellular rejection in the viable skin of the TCDD-treated mice (Figure 1C). In contrast, skin grafts from untreated mice exhibited significant necrosis with severe mononuclear cellular infiltrate on POD 10 and were fully necrotic on POD 16.

To understand a possible mechanism of skin graft prolongation, splenic T cells were analyzed on POD 10 by flow cytometry. Elevated levels of FoxP3⁺ CD4⁺ T cells were present in mice treated with TCDD, independent of whether the animal had received a syngeneic or an allogeneic skin graft (Figure 1D) (E). A similar result was noted in mice treated with TCDD without skin grafts. Expression of FoxP3 was found to be elevated 7 days after TCDD treatment in the spleen, lymph node, thymus and blood compared to untreated mice (Supplemental Figure 2). The marker Ki67 was also used as an indicator of T-cell proliferation *in vivo*. Both treated and untreated mice receiving allogeneic skin grafts had higher levels of Ki67 expression in both CD4⁺ and CD8⁺ T-cell subsets compared to syngeneic recipients (Figure 1E). TCDD treatment resulted in suppression of Ki67 expression in CD8⁺ T cells compared to untreated controls, while in CD4⁺ T cells the effect was not significant.

Exploration of a possible mechanism behind TCDD-mediated allograft prolongation

Naïve CD4⁺ T cells from the spleens of B6 mice or AHR^{-/-} mice were stimulated with soluble CD3 and CD28 antibodies in RPMI for 5 days in the presence of TCDD, FICZ or TGF- β . At the end of the culture period, cells were harvested and RNA was extracted for analysis of FoxP3 expression by qPCR. In contrast to cells activated in the presence of TGF- β , naïve T cells activated in the presence of TCDD or FICZ did not demonstrate a significant change in FoxP3 expression (Figure 2A). AHR^{-/-} T cells showed less FoxP3 upregulation with TGF- β , and no change with either TCDD or FICZ. This requirement of AHR expression for optimal FoxP3 expression by TGF- β speaks to the important role of the AHR in T-cell differentiation, and was further characterized in a previous publication (Mezrich *et al.* 2010).

To determine IDO expression in DC, BMDC from either B6 or AHR^{-/-} mice were placed in culture in the presence of IFN- γ , the TLR9 ligand CpG, TCDD or FICZ. After 5 days of culture, cells were harvested and RNA was analyzed for IDO mRNA expression. Data demonstrated that IFN- γ led to IDO induction in both AHR^{-/-} and wild-type cells (Figure

2B). In contrast, TCDD and FICZ both resulted in IDO induction in BMDC of B6 mice but not from AHR^{-/-} mice.

Utilizing an *in vitro* system, naive CD4⁺ T cells were isolated from B6 splenocytes and then stimulated by pDC, which were isolated from spleen of BALB/c mice. This model was previously shown to generate Tregs in an IDO-dependant manner (W. Chen *et al.* 2008). After 5 days of culture in the presence of TCDD, FICZ or vehicle, cells were harvested and RNA was analyzed for CYP1A1 (a marker of AHR activation), IDO, FoxP3 and TGF- β mRNA expression. As expected, the addition of either TCDD or FICZ led to CYP1A1 up-regulation (data not shown). Furthermore, the presence of TCDD in the co-culture led to up-regulation of both IDO and FoxP3, but FICZ did not (Figure 2C). TCDD did not significantly up-regulate TGF- β mRNA levels (data not shown).

FICZ accelerates fully mismatched skin graft rejection in mice

B6 recipient mice were treated with FICZ IP on POD -1 and 4 and compared to animals that received no treatment or DMSO-treated controls. Animals were followed and sacrificed when the grafts were deemed fully rejected. As seen in the survival curve (Figure 3A), mice treated with FICZ experienced accelerated rejection compared to control animals. There was no marked difference between control groups. This was demonstrated in the pathology (Figure 3B) showing a rejected skin graft from a recipient mouse treated with FICZ on POD 9 which is contrasted with control that indicated some viable skin at this time point. Cytokine expression of recipient splenocytes (POD10) following a 3-day stimulation with irradiated-syngeneic or allogeneic splenocytes revealed less expression of IFN- γ but greater expression of IL-17 in the culture supernatant from splenocytes of FICZ-treated recipients (Figure 3C).

AHR^{-/-} mice do reject fully mismatched skin grafts

AHR^{-/-} mice on a B6 background were recipients of Balb/c skin grafts and graft survival was compared to wild-type recipients, all with no treatment. As seen in Figure 4A, while the nulls seemed to trend towards numerically longer survival, this did not reach significance, and ultimately their mean rejection times in this fully-mismatched model were not significantly different than controls. Histological examination on POD 9 showed mild cellular infiltrate with viable skin (Figure 4B) while histology on POD 13 demonstrated complete rejection (data not shown). Flow cytometric analysis of splenocytes isolated on POD 10 revealed that AHR^{-/-} recipients had less proliferation *in vivo* of both CD4⁺ and CD8⁺ T cells (Figure 4C). Stimulation of AHR^{-/-} splenocytes with irradiated Balb/c splenocytes resulted in significantly less IFN- γ in culture supernatants compared to wild-type mice (Figure 4D). IL-17 secretion, although quantitatively less, was not significantly decreased.

DISCUSSION

This study confirmed that TCDD treatment prolongs fully mismatched skin grafts, possibly by enhancing the Treg response via IDO up-regulation. In contrast, treatment with FICZ accelerates skin graft rejection. This represents one of the first examples highlighting the complex nature of the interaction between the AHR and the immune response to alloantigens. Previous data indicated that TCDD treatment enhanced Treg induction *in vivo* (Quintana *et al.* 2008). The increase in FoxP3⁺ T cells *in vivo* following TCDD treatment was seen in animal models of Crohn's disease (Benson and Shepherd 2010), diabetes (Kerkvliet *et al.* 2009) and uveoretinitis (Zhang *et al.* 2010). TCDD alone *in vitro* did not influence differentiation of naïve T cells with antibody stimulation in the study, but required the presence of DC to enhance Treg development. This may be secondary to IDO generation

by the DC in response to activation of the AHR in these cells. Previously, Mezrich et al (2010) showed that kynurenine, an AHR ligand, led to Treg generation from naïve T cells directly, in an AHR-dependent fashion, which was enhanced with the presence of DC. Fallarino et al (2006) previously noted that kynurenine resulted in generation of Tregs from naïve T cells as long as low Trp media was utilized, but not when higher Trp media was used. It may be that when high levels of Trp are present, it is preferentially taken up by the T cell via a transporter that exchanges Trp and kynurenine, preventing adequate kynurenine to enter the cell and interact with the AHR. This competitive uptake was proposed in previous studies examining L-amino acid transporters (Kaper *et al.* 2007, Nii *et al.* 2001). In this model, when DC are present to generate IDO, DC metabolize and deplete the Trp, allowing kynurenine to enter the T cell and enhance Treg generation. When DC are not present and high-Trp media is used, the effect of kynurenine on T cells is mitigated. TCDD generates IDO in DC via the AHR, and this may drive the DC-dependent Treg differentiation in response to this toxicant.

A single receptor controlling the generation of Treg, or alternatively Th17 cells, would be beneficial physiologically because in the setting of an immunologic insult in an organism, as the balance of inflammatory cytokines and AHR ligands increased, more Th17 cells would be generated, and at the same time fewer Tregs, allowing the inflammatory response needed to control the insult. Once the situation is controlled, the balance needs to shift, favoring differentiation of regulatory over effector cells and dissipation of the immune response. Figure 5 represents our model of the role the AHR might play in DC-T cell interactions. Depending on the balance of Trp, FICZ, and kynurenine, differentiation of T cells favor an effector or a regulatory response. This model represents a new paradigm in understanding T-cell differentiation.

The above discussion relates to the endogenous role of the AHR during an immune response. In addition, the AHR clearly has a role in responding to environmental exposures and toxicants, and it contains a PAS protein domain, a family of proteins that has evolved to respond to environmental signals (Taylor and Zhulin 1999). It is possible this receptor may be a sensor for various environmental exposures, many of them harmful. Its activation leads to transcription of cytochrome P450 enzymes, which serve to metabolize many of these ligands, to minimize their toxicity and protect the host organism. TCDD and kynurenine led to an enhancement of regulatory T cells, and only FICZ enhanced Th17 development. Attempts over the last couple of years to identify other ligands that also enhance Th17 differentiation were without success. More recently evidence indicates that FICZ, which is generated by UV light exposure in skin, might itself be a signal of environmental exposure to excessive, harmful sunlight. This compromises mucosal defenses and natural immunity, allowing invasion by endogenous bacteria that normally are not pathogenic. For this reason, a link between AHR, which senses these environmental signals, and the acquired immune system, might provide an evolutionary advantage. Specifically, activation of the AHR by pathogenic toxicants results in an increase in Th17 cell differentiation to help compensate for decreased mucosal immunity and susceptibility to endogenous infectious agents in these areas. The exposure to additional environmental factors like diesel exhaust, urban dust, and cigarette smoke, all of which have components (polycyclic aromatic hydrocarbons, PAH) that activate the AHR, compromise mucosal defenses and natural immunity in the lung, a location that contains endogenous bacteria that normally are not pathogenic. There have been reports of exposing murine lungs to mixtures including PAH. In one study, urban dust particles collected and injected intratracheally in mice (Saunders *et al.* 2010) demonstrated that the ensuing airway hyperresponsiveness was (1) lymphocyte dependent, (2) there was recruitment of CD4⁺ T cells to the airways, and (3) high levels of Th17-associated cytokines were identified in bronchoalveolar lavages. In a second study, cigarette smoke extract

avored Th17 differentiation in naïve T cells, both *in vitro* and *in vivo*, in an AHR-dependent manner (K. Chen *et al.* 2011).

While the above discussion suggests that the relationship between the acquired immune system and the AHR is of evolutionary benefit, in the unique circumstance of organ transplantation this relationship might serve as a detriment, with various environmental exposures leading to increased rejection. Further investigations on the role that environmental ligands might play in graft survival after transplantation are planned. Pinpointing specific exposures that may already be leading to loss of transplanted organs, may help design new strategies in the form of avoidance or drugs targeting the AHR to improve transplant outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by Grant 1UL1RR025011 from the Clinical and Translational Science Award program of the National Center for Research Resources, National Institutes of Health (J.D.M.), National Institutes of Environmental Health Services Grant R37ES005703 (C.A.B.), National Cancer Institute Grant P30CA014520 (C.A.B.), an American Society of Transplant Surgeons-Astellas Faculty Development Award (J.D.M.). J.D.M. is a John Merrill Grant Scholar of the American Society of Nephrology.

Abbreviations

AHR	aryl hydrocarbon receptor
BMDC	bone marrow-derived dendritic cell
DC	dendritic cell
EAE	experimental autoimmune encephalitis
FICZ	6-formylindolo[3,2-b]carbazole
IDO	indoleamine 2,3-dioxygenase
PAHs	polycyclic aromatic hydrocarbons
pDC	plasmacytoid dendritic cell
POD	post-operative days
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
Treg	FoxP3 ⁺ regulatory T cells
Trp	tryptophan
Cyp1A1	Cytochrome P450 1A1

REFERENCES

- Bankoti J, Rase B, Simones T, Shepherd DM. Functional and phenotypic effects of ahr activation in inflammatory dendritic cells. *Toxicology and Applied Pharmacology*. 2010; 246(no. 1–2):18–28. [PubMed: 20350561]
- Benson JM, Shepherd DM. Aryl hydrocarbon receptor activation by tcdd reduces inflammation associated with crohn's disease. *Toxicological Sciences*. 2010
- Chen K, Pociask DA, Mcaleer JP, Chan YR, Alcorn JF, Kreindler JL, Keyser MR, Shapiro SD, Houghton AM, Kolls JK, Zheng M. Il-17ra is required for ccl2 expression, macrophage recruitment,

- and emphysema in response to cigarette smoke. *PLoS One*. 2011; 6(no. 5):e20333. [PubMed: 21647421]
- Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive t regulatory cell generation. *The Journal of Immunology*. 2008; 181(no. 8):5396–5404. [PubMed: 18832696]
- Diani-Moore S, Labitzke E, Brown R, Garvin A, Wong L, Rifkind AB. Sunlight generates multiple tryptophan photoproducts eliciting high efficacy cyp1a induction in chick hepatocytes and in vivo. *Toxicological Sciences*. 2006; 90(no. 1):96–110. [PubMed: 16330490]
- Fallarino F, Grohmann U, You S, Mcgrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC, Puccetti P. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate t cell receptor zeta-chain and induce a regulatory phenotype in naive t cells. *The Journal of Immunology*. 2006; 176(no. 11):6752–6761. [PubMed: 16709834]
- Fritsche E, Schafer C, Calles C, Bernsmann T, Bernshausen T, Wurm M, Hubenthal U, Cline JE, Hajimiragha H, Schroeder P, Klotz LO, Rannug A, Furst P, Hanenberg H, Abel J, Krutmann J. Lightening up the uv response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet b radiation. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(no. 21):8851–8856. [PubMed: 17502624]
- Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of t and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *The Journal of Experimental Medicine*. 2002; 196(no. 4):459–468. [PubMed: 12186838]
- Johnson ES, Parsons W, Weinberg CR, Shore DL, Mathews J, Patterson DG Jr, Needham LL. Current serum levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in phenoxy acid herbicide applicators and characterization of historical levels. *Journal of the National Cancer Institute*. 1992; 84(no. 21):1648–1653. [PubMed: 1433346]
- Jux B, Kadow S, Esser C. Langerhans cell maturation and contact hypersensitivity are impaired in aryl hydrocarbon receptor-null mice. *The Journal of Immunology*. 2009; 182(no. 11):6709–6717. [PubMed: 19454665]
- Jux B, Kadow S, Luecke S, Rannug A, Krutmann J, Esser C. The aryl hydrocarbon receptor mediates uvb radiation-induced skin tanning. *The Journal of Investigative Dermatology*. 2011; 131(no. 1):203–210. [PubMed: 20861855]
- Kaper T, Looger LL, Takanaga H, Platten M, Steinman L, Frommer WB. Nanosensor detection of an immunoregulatory tryptophan influx/kynurenine efflux cycle. *PLoS Biology*. 2007; 5(no. 10):e257. [PubMed: 17896864]
- Kerkvliet NI, Steppan LB, Vorachek W, Oda S, Farrer D, Wong CP, Pham D, Mourich DV. Activation of aryl hydrocarbon receptor by tcdd prevents diabetes in nod mice and increases foxp3+ t cells in pancreatic lymph nodes. *Immunotherapy*. 2009; 1(no. 4):539–547. [PubMed: 20174617]
- Mellor AL, Munn DH. Ido expression by dendritic cells: Tolerance and tryptophan catabolism. *Nature Reviews. Immunology*. 2004; 4(no. 10):762–774.
- Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory t cells. *The Journal of Immunology*. 2010; 185(no. 6):3190–3198. [PubMed: 20720200]
- Nair S, Kekatpure VD, Judson BL, Rifkind AB, Granstein RD, Boyle JO, Subbaramaiah K, Guttentplan JB, Dannenberg AJ. Uvr exposure sensitizes keratinocytes to DNA adduct formation. *Cancer Prevention Research (Philadelphia)*. 2009; 2(no. 10):895–902.
- Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(no. 46):19961–19966. [PubMed: 21041655]
- Nii T, Segawa H, Taketani Y, Tani Y, Ohkido M, Kishida S, Ito M, Endou H, Kanai Y, Takeda E, Miyamoto K. Molecular events involved in up-regulating human na+-independent neutral amino acid transporter lat1 during t-cell activation. *Biochemical Journal*. 2001; 358(no. Pt 3):693–704. [PubMed: 11535130]

- Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL. Control of t(reg) and t(h)17 cell differentiation by the aryl hydrocarbon receptor. *Nature*. 2008; 453(no. 7191):65–71. [PubMed: 18362915]
- Quintana FJ, Murugaiyan G, Farez MF, Mitsdoerffer M, Tukpah AM, Burns EJ, Weiner HL. An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and t cells to suppress experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(no. 48):20768–20773. [PubMed: 21068375]
- Rannug U, Rannug A, Sjoberg U, Li H, Westerholm R, Bergman J. Structure elucidation of two tryptophan-derived, high affinity ah receptor ligands. *Chemistry & Biology*. 1995; 2(no. 12): 841–845.
- Saunders V, Breyse P, Clark J, Sproles A, Davila M, Wills-Karp M. Particulate matter-induced airway hyperresponsiveness is lymphocyte dependent. *Environmental Health Perspectives*. 2010; 118(no. 5):640–646. [PubMed: 20061214]
- Schmidt JV, Carver LA, Bradfield CA. Molecular characterization of the murine ahr gene. Organization, promoter analysis, and chromosomal assignment. *The Journal of Biological Chemistry*. 1993; 268(no. 29):22203–22209. [PubMed: 8408082]
- Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine ahr null allele: Involvement of the ah receptor in hepatic growth and development. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93(no. 13):6731–6736. [PubMed: 8692887]
- Simones T, Shepherd DM. Consequences of ahr activation in steady-state dendritic cells. *Toxicological Sciences*. 2011; 119(no. 2):293–307. [PubMed: 21097750]
- Taylor BL, Zhulin IB. Pas domains: Internal sensors of oxygen, redox potential, and light. *Microbiology and Molecular Biology Reviews*. 1999; 63(no. 2):479–506. [PubMed: 10357859]
- Terness P, Bauer TM, Rose L, Dufter C, Watzlik A, Simon H, Opelz G. Inhibition of allogeneic t cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: Mediation of suppression by tryptophan metabolites. *The Journal of Experimental Medicine*. 2002; 196(no. 4):447–457. [PubMed: 12186837]
- Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links t(h)17-cell-mediated autoimmunity to environmental toxins. *Nature*. 2008; 453(no. 7191):106–109. [PubMed: 18362914]
- Vogel CF, Goth SR, Dong B, Pessah IN, Matsumura F. Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochemical and Biophysical Research Communications*. 2008; 375(no. 3):331–335. [PubMed: 18694728]
- Wei YD, Bergander L, Rannug U, Rannug A. Regulation of cyp1a1 transcription via the metabolism of the tryptophan-derived 6-formylindolo[3,2-b]carbazole. *Archives of Biochemistry and Biophysics*. 2000; 383(no. 1):99–107. [PubMed: 11097181]
- Zhang L, Ma J, Takeuchi M, Usui Y, Hattori T, Okunuki Y, Yamakawa N, Kezuka T, Kuroda M, Goto H. Suppression of experimental autoimmune uveoretinitis by inducing differentiation of regulatory t cells via activation of aryl hydrocarbon receptor. *Investigative Ophthalmology & Visual Science*. 2010; 51(no. 4):2109–2117. [PubMed: 20007828]

Figure 1.

A single dose of TCDD delays Balb/c skin graft rejection in C57BL/6 mice. (A) C57BL/6 mice (n=6) were injected with a single dose of TCDD one day prior to receiving a Balb/c skin graft. Mice were sacrificed on POD 10 (n=2), POD 16 (n=2) or at the time of skin graft rejection. (B) Treatment with TCDD (n = 8; $p < 0.05$). (C) Skin allografts taken from untreated or TCDD-treated recipient mice on POD10 or POD 16 were stained using a standard H&E protocol for histological examination. Transplanted skin is designated by A and native skin is designated by B. (D) Histograms depicting FoxP3 expression as determined by flow cytometry of CD3⁺ CD4⁺ splenocytes isolated on POD 10 from syngeneic or allogeneic skin graft recipient mice that either received no treatment or TCDD. (E) Splenocytes were isolated from syngeneic or allogeneic skin graft recipient mice on POD 10. The % CD4⁺ T cells positive for FoxP3 and Ki67 and % CD8⁺ T cells positive for Ki67 were determined by flow cytometry.

Figure 2.

Actions of AHR activation by TCDD and FICZ *in vitro*. (A) Naive B6 T cells were cultured with CD3 and CD28 mAB alone or in the presence of either TGF-β (4ng/ml), TCDD (10nM) or FICZ (100 nM). mRNA was isolated on day 5 and qPCR was performed. Expression of FoxP3 was measured in treated cells relative to untreated cells. (B) BMDC were generated from the bone marrow of B6 mice. Cells were harvested on day 6 as immature BMDC. BMDC were then cultured alone or in the presence of IFN-γ (100ng/ml), CpG (1μM), TCDD (10nM) or FICZ (100nM). After 2 days, mRNA was isolated and qPCR was used to assay for the expression of IDO1 in treated BMDC versus untreated BMDC. (C) Naive CD4⁺ CD25⁻ T cells were isolated from B6 splenocytes and co-cultured with pDC

isolated from Balb/c mice at a 10:1 T cell to pDC ratio alone or in the presence of TCDD (10nM) or FICZ (100nM). On day 5, cells were harvested and mRNA was isolated for qPCR analysis. Expression of IDO1 and FoxP3 was measured in treated cells relative to untreated cells. Data was analyzed by ANOVA and Bonferroni Multiple Comparison. * $p < 0.05$. Data shown are representative of four experiments.

Figure 3.

Treatment with FICZ accelerates Balb/c skin graft rejection in C57BL/6 mice. (A) C57BL/6 mice (n=12) were treated with FICZ one day prior to and 4 days after receiving a Balb/c skin graft. Treatment with FICZ (Median survival time (MST = 8) skin graft rejection compared to untreated mice (n = 7; MST = 11; p < 0.05) or diluent-treated mice (n=5; MST = 11; p<0.05). (B) Skin allografts taken from untreated or FICZ-treated recipient mice on POD 9 were stained for histological examination using a standard H&E protocol. Transplanted skin is designated by A and native skin is designated by B. Necrotic skin from FICZ-treated mice represents rejected graft while the scab on the untreated graft is superficial to viable epithelium. (C) Splenocytes from naïve mice, untreated mice receiving a Balb/c skin graft and FICZ-treated mice receiving a Balb/c skin graft were stimulated *in vitro* for 3 days with irradiated B6 or Balb/c splenocytes. Culture supernatants were harvested and IFN- γ and IL-17 concentration was measured by cytokine bead array.

Figure 4.

Lack of AHR expression does not significantly alter Balb/c skin graft survival in C57BL/6 mice. (A) AHR^{-/-} mice with a C57BL/6 background (n=8) received a Balb/c skin graft and the MST was 12.5 days. (n = 7; MST = 11). (B) Skin allografts taken from wild-type B6 or AHR^{-/-} recipient mice on POD 9 were stained for histological examination using a standard H&E protocol. (C) Splenocytes were isolated from B6 wild-type or AHR^{-/-} mice recipients of allogeneic skin grafts on POD 9. The % CD4⁺ T cells positive for FoxP3 and Ki67 and % of CD8⁺ T cells positive for Ki67 were determined by flow cytometry. (D) Splenocytes from naive mice, B6 wild-type mice receiving a Balb/c skin graft and AHR^{-/-} mice receiving a Balb/c skin graft were stimulated *in vitro* for 3 days with irradiated B6 or Balb/c splenocytes. Culture supernatants were harvested and IFN- γ and IL-17 concentration was measured by cytokine bead array.

Figure 5. DC/T cell interactions in T-cell differentiation. IFN- γ or AHR ligand leads to IDO upregulation in DC, which generate other ligands (kynurenine) that activate the AHR in T cells enhancing Treg differentiation. In DC that have little to no IDO activity, ligands like FICZ are produced, favoring TH17 differentiation.