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The aryl hydrocarbon receptor: a perspective on potential roles in the immune system

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Summary

The aryl hydrocarbon receptor (AHR) is a protein best known for its role in mediating toxicity. Over 30 years of research has uncovered additional roles for the AHR in xenobiotic metabolism and normal vascular development. Activation of the AHR has long been known to cause immunotoxicity, including thymic involution. Recent data suggesting a role for the AHR in regulatory T-cell (Treg) and T-helper 17 (Th17) cell development have only added to the excitement about this biology. In this review, we will attempt to illustrate what is currently known about AHR biology in the hope that data from fields as diverse as evolutionary biology and pharmacology will help elucidate the mechanism by which AHR modifies immune responses. We also will discuss the complexities of AHR pharmacology and genetics that may influence future studies of AHR in the immune system.

Keywords: aryl hydrocarbon receptor; dioxin; immune function; thymus; T lymphocytes

Introduction

The aryl hydrocarbon receptor (AHR) is a protein of ancient origins. Phylogenetic analysis reveals that functional orthologues of the *Ahr* gene are present in living mammals, amphibians, reptiles and birds.[1] For more than 30 years, the AHR has been studied as a receptor for environmental contaminants and as a mediator of chemical toxicity. Recently, an additional role for the AHR in normal vascular development has been identified. Long-standing literature on 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) toxicology, as well as a flurry of recent high-profile papers, has suggested a role for this protein in immunology. In this review, we will provide an overview of AHR signal transduction with an emphasis on providing information that may guide future studies aimed at delineating the role of this protein in human immunity and related disease.

The AHR signalling pathway

The AHR is a ligand-activated transcription factor from the Per-Arnt-Sim (PAS) superfamily of proteins.[2] Analysis of the AHR reveals an N-terminal basic helix loop helix (bHLH) domain, a C-terminal variable domain, and a central PAS domain with two degenerate repeats (denoted repeat A and repeat B).[3–5] The PAS domain of the AHR mediates heterodimerization with a structurally related protein known as the aryl hydrocarbon receptor nuclear translocator (ARNT), DNA recognition, ligand binding

Abbreviations: AHR, aryl hydrocarbon receptor; AHRR, aryl hydrocarbon receptor repressor; ARA9, aryl hydrocarbon receptor associated 9; ARNT, aryl hydrocarbon receptor nuclear translocator; BaP, benzo-a-pyrene; bHLH, basic helix loop helix; Cre-Lox, cre recombinase mediated LoxP site recombination; CYP, cytochrome P450; DC, dendritic cell; DRE, dioxin response element; EAE, experimental autoimmune encephalitis; FICZ, 6-formylindolo(3,2-*b*)carbazole; FoxP3, forkhead box P3; GVHD, graft versus host disease; HAH, halogenated aryl hydrocarbon; Hsp90, heat shock protein 90; ICZ, indolo(3,2-*b*)carbazole; ITE, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester; K_D, dissociation constant; *Klf2*, Kruppel like factor 2; LDL, low density lipoprotein; NES, nuclear export signal; NLS, nuclear localization signal; Nrf2, nuclear erythroid 2-related factor; p23, protein 23; PAH, polycyclic aromatic hydrocarbon; PAS, Per-ARNT-Sim; PCB, polychlorinated biphenyl; pentaCB, penta chlorinated byphenyls; RIP140, receptor interacting protein 140; SHP, nuclear receptor subfamily 0 group B member 2; Sp1, transacting transcription factor 1; SRC, nuclear receptor coactivator; TAD, transcriptionally active domain; TCDD, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin; Th17, T-helper 17; Treg, regulatory T cell; UGT1A6, UDP glucuronosyltransferase 1 family polypeptide A6.

and chaperone interactions.[5–7] Next to the PAS domain is a bHLH domain that is involved in DNA binding and support of dimerization.[4,6,8] The C-terminal half is highly variable and responsible for differences in receptor molecular weight within and across species.[2,5] The largely unstructured C-terminal region contains a transcriptionally active domain and potentially domains involved in receptor transformation[2,5] (Fig. 1).

In the absence of bound agonist, the AHR is most commonly found in the cytoplasm in a complex with its chaperones heat shock protein 90 (Hsp90), P23 and aryl hydrocarbon receptor associated 9 (ARA9; aka AIP,

XAP2).[9,10,12] In addition to holding the receptor in a form able to bind ligand, Hsp90 prevents surreptitious nuclear translocation of the AHR.[9] The cochaperone p23 stabilizes the AHR–Hsp90 interaction, while the ARA9 protein enhances AHR signalling by increasing the amount of properly folded AHR in the cytoplasm.[10,12] Upon agonist binding, the AHR–chaperone complex translocates to the nucleus and binds the ARNT protein.[13,14] The ARNT protein is structurally similar to the AHR (Fig. 1). This heterodimeric pairing yields a competent transcription factor within the nuclear compartment of cells (Fig. 2).
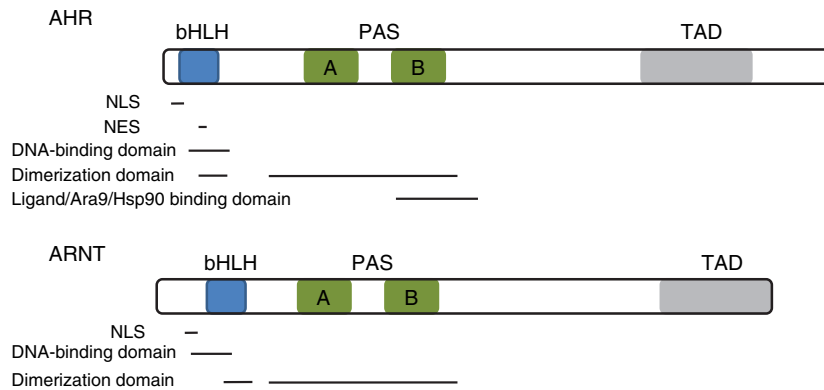

Figure 1. Protein domains found in the aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT). The nuclear localization sequence (NLS) and nuclear export sequence (NES) are found within the basic helix loop helix (bHLH) region. The bHLH also plays a critical role in DNA binding. The characteristic domains in Per-ARNT-Sim (PAS) family members mediate heterodimerization and chaperone binding. The C-terminus is variable, but contains the transactivation domain (TAD) responsible for activating transcription after DNA binding.
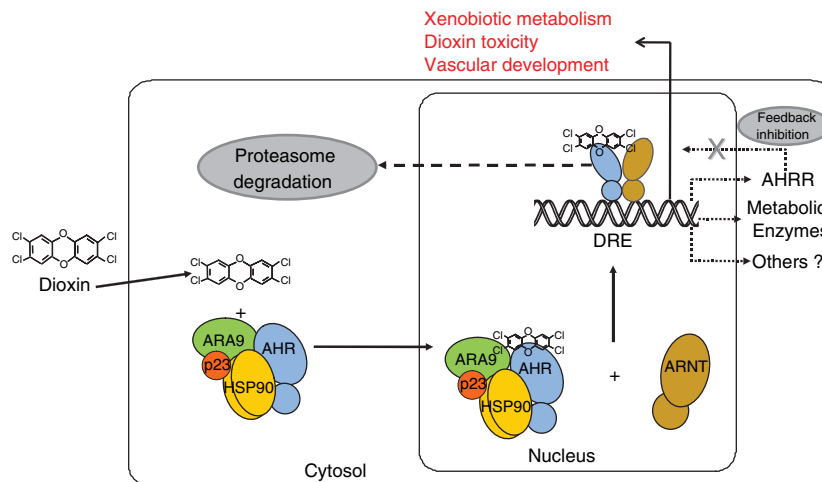

Figure 2. The aryl hydrocarbon receptor (AHR) signalling pathway. Hydrophobic ligands enter the cell via diffusion through the cell membrane. Ligands bind to the AHR in the cytosol. Ligand binding causes conformational changes leading to nuclear localization sequence (NLS) exposure and the AHR complex translocates to the nucleus. In the nucleus, the AHR binds its heterodimeric partner, the aryl hydrocarbon receptor nuclear translocator (ARNT) and directs transcription from dioxin response elements (DREs), upstream of target genes. Signalling through the AHR is down-regulated by two means, the proteasome and a feed-back pathway involving the aryl hydrocarbon receptor repressor (AHRR). The AHRR is an AHR gene target and its expression is up-regulated by AHR signalling. Signalling by the AHR leads to three biological pathways referred to as the adaptive metabolic pathway, the toxic pathway, and the developmental pathway. ARA9, aryl hydrocarbon receptor associated 9; Hsp90, heat shock protein 90.

The AHR:ARNT heterodimer directs transcription of genes from dioxin-responsive enhancer elements (DREs) within the genome. These classic enhancers are found near AHR transcriptional targets. These primary targets are commonly known as the 'AHR gene battery'. Regulation of this gene battery has been shown to be dependent upon a number of common coactivators. For example, histone acetyl transferases are recruited to the DRE-regulated promoters through coactivators such as nuclear receptor coactivator (SRC) and p300.[15–17] Additionally, transcriptional cofactors, such as transacting transcription factor 1 (Sp1), receptor interacting protein 140 (RIP140), and nuclear receptor subfamily 0 group B member 2 (SHP), activate the transcriptional response of the AHR:ARNT heterodimer.[18,19] Although the list of members of the AHR gene battery is still expanding, it includes those that encode xenobiotic metabolizing enzymes, such as cytochromes P450 (CYP) 1A1, CYP1A2, CYP1B1 and UDP glucuronosyltransferase 1 family polypeptide A6 (UGT1A6)[20] (Table 1). Collectively these enzymes are well known for their important roles in the clearance of foreign chemicals.

Signalling through the AHR can be down-regulated by at least two means. Upon entering the nucleus, the ligand-activated AHR is exported and degraded by the ubiquitin/proteosome pathway.[21] Like many other PAS signalling pathways, AHR signalling includes a negative feedback arm. Signalling by the AHR is attenuated by another PAS protein known as the AHR repressor (AHRR). The AHRR is a DRE-regulated gene and its expression increases rapidly upon AHR activation[22] (Fig. 2). The AHRR is structurally similar to the AHR, but contains a potent transcriptional repressor domain and does not require an agonist to dimerize with ARNT. Down-regulation of AHR signalling by these two independent means implies that there is evolutionary selection against overactivation of the AHR gene battery.

AHR-mediated biology

The adaptive pathway

The AHR was originally characterized as a regulator of xenobiotic metabolism, specifically that of polycyclic aromatic hydrocarbons (PAH). Early experiments revealed that exposure to pollutants such as benzo[a]pyrene led to marked increases in cytochromes P450 that act to hydroxylate this foreign chemical, increasing its water solubility and decreasing its biological residency.[23] This pathway fits the definition of an 'adaptive metabolic response', in that cytosolic AHR binds xenobiotic ligands and activates transcription of enzymes that mediate their biotransformation and excretion.[24]

The toxic pathway

In response to halogenated dibenzo-p-dioxins ('dioxins') and related biphenyls and dibenzofurans, AHR activation not only induces the adaptive xenobiotic metabolic pathway, but also mediates a variety of toxic responses. Dioxin toxicity commonly includes hepatocellular damage, thymic involution, immune suppression, chloracne, epithelial hyperplasia, teratogenesis, and tumour promotion.[25–28] Evidence to support the role of the AHR in toxicity is twofold. First, the binding affinity of dioxin congeners for the AHR corresponds to their toxic potency in vivo.[29] For example, the ligand TCDD displays the greatest affinity for the AHR and is the most toxic, while the weaker 2,8-dichloro congener has a 300-fold lower affinity and is essentially non-toxic.[26,29] Secondly, mice harbouring the Ahr^b allele, which codes for a receptor with a high binding affinity for agonists, display an increased incidence of dioxin toxicity and induction of AHR battery genes compared with mice harbouring the Ahr^d allele, which encodes a receptor with a 10-fold lower affinity.[30–32] In addition to binding affinities with dissociation constant (K_D) values in the picomolar range, TCDD is not appreciably metabolized, thus causing prolonged AHR activation.[32] Because of its remarkable potency and biological stability, TCDD has proved to be invaluable in elucidating the mechanism of AHR signalling and enzyme induction.

The developmental pathway

To elucidate the physiological and developmental importance of the AHR, several laboratories have characterized Ahr null mice.[33–35] These models differ in some respects, yet display key in vivo phenotypic similarities.[36] As expected, in response to PAHs and dioxins, Ahr null mice cannot up-regulate the metabolic enzymes characteristic of the adaptive pathway.[33–35] Additionally, these animals are resistant to most, if not all, aspects of dioxin toxicity.[35] Indicating a role in normal physiology, a number of surprising pathologies also have been characterized in these mouse models. For example, Ahr null mice have markedly smaller livers than wild-type littermates and have abnormal vasculature in the liver, kidney and eye.[33,34,37] Abnormal hepatic circulation, characterized by anastomotic sinusoidal vessels, appears to cause decreased perfusion and necrosis of the liver periphery.[37,38] This can be demonstrated in Ahr null embryos as early as embryonic day 15.[37,38] In addition, extramedullary haematopoiesis, fatty metamorphosis of hepatocytes, and portal tract fibrosis have been observed in Ahr null livers.[33,34] Cardiac hypertrophy, hypertension, and elevated levels of vasoconstrictors are also seen in Ahr null animals.[39]

Table 1. Aryl hydrocarbon receptor (AHR)-regulated genes. The genes listed here were identified as differentially expressed upon 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin exposure. All samples were compared to vehicle-treated control samples. The official gene symbol, gene name and Gen-Bank accession number are provided. To search for dioxin responsive enhancer (DRE) sequences, we pulled the upstream sequence for each gene from the University of California–Santa Cruz (UCSC) genome browser and searched for DRE sequences on either the + or − strand using Motif-Viz.[112,113] The number of times the DRE consensus sequence, TNGCGTG, was identified within 15 000 bases upstream of the gene target is indicated in the DRE column. In the Gene expression column, ↑ indicates that the gene was upregulate by TCDD treatment. A ↓ indicates that the expression of the gene was downregulated by TCDD. The number in the Gene expression column indicates the fold change between the TCDD and vehicle control treated samples. The Cell type column indicates the cell population used in each published experiment. Double negative-recent thymic emigrants (DN-RTE), triple negative X T-cell population (TNX), graft versus host disease (GVHD).

Gene symbol	Gene name	Genebank accession	DRE	Gene expression	Cell type	Reference
Cyp1a1	CYP450, family 1, subfamily a, polypeptide 1	NM_001136059.1	5	38↑	Liver	[114]
Fabp5	Fatty acid binding protein 5	NM_010634.2	0	4↑	Liver	[114]
Gsta2	Glutathione S-transferase α2	NM_008182.3	3	7↑	Liver	[114]
Itgb1	Integrin β 1	NM_010578.1	0	3↑	Liver	[114]
Notch1	Notch homolog 1	NM_008714.2	4	3↑	Liver	[114]
Nqo1	NAD(P)H dehydrogenase, quinone 1	NM_008706.5	4	5↑	Liver	[114]
Ugdh	UDP-glucose dehydrogenase	NM_009466.2	2	3↑	Liver	[114]
Car3	Carbonic anhydrase 3	NM_007606.3	1	4↓	Liver	[115]
Cdca5	Cell division cycle associated 5	NM_026410.3	3	9↑	Liver	[115]
Cfdp1	Craniofacial development protein 1	NM_011801.1	3	4↑	Liver	[115]
Epyc	Epiphycan	NM_007884.2	1	6↑	Liver	[115]
Gadd45b	Growth arrest & DNA damage-inducible 45β	NM_008655.1	2	5↑	Liver	[115]
Lpin2	Lipin2	NM_022882.3	2	3↑	Liver	[115]
Mrpl37	Mitochondrial ribosomal protein L37	NM_025500.1	3	8↑	Liver	[115]
Myc	Myelocytomatosis oncogene	NM_010849.4	2	4↑	Liver	[115]
Pabpc2	Poly(A) binding protein, cytoplasmic 2	NM_011033.2	0	7↑	Liver	[115]
Pak1ip1	PAK1 interacting protein 1	NM_026550.2	1	4↑	Liver	[115]
Tiparp	TCDD-inducible poly(ADP-ribose) polymerase	NM_178892.5	0	10↑	Liver	[115]
Tnfaip2	Tumor necrosis factor, α-induced protein 2	NM_009396.2	4	6↑	Liver	[115]
Ahrr	AHR repressor	NM_009644.2	2	21↑	CD4+ cells (GVHD)	[72]
Ccr4	Chemokine (C-C motif) recptor 4	NM_009916.2	4	5↑	CD4+ cells (GVHD)	[72]
Ccr5	Chemokine (C-C motif) recptor 5	NM_009917.5	0	3↑	CD4+ cells (GVHD)	[72]
Ccr9	Chemokine (C-C motif) recptor 9	NM_009913.5	3	5↑	CD4+ cells (GVHD)	[72]
Gzmb	Granzyme B	NM_013542.2	1	6↑	CD4+ cells (GVHD)	[72]
IL12rb2	Interleukin 12 receptor, β2	NM_008354.3	1	10↑	CD4+ cells (GVHD)	[72]
Prdm1	PR domain containing 1	NM_007548.3	2	3↑	CD4+ cells (GVHD)	[72]
Stat4	Signal transducer & activator of transcription 4	NM_011487.4	2	3↑	CD4+ cells (GVHD)	[72]
Tgfb3	Transforming growth factor β3	NM_009368.2	0	13↑	CD4+ cells (GVHD)	[72]
Acpp	Acid phosphatase, prostate	NM_207668.2	4	16↑	TNX	[66]
Bcl9	B-cell lymphoma 9	NM_029933.3	3	20↑	TNX	[66]
Ctxn1	Cortexin 1	NM_183315.2	2	12↑	TNX	[66]
Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	NM_010501.2	3	10↑	TNX	[66]
IL12rb1	Interleukin 12 receptor β1	NM_008353.2	1	13↑	TNX	[66]
Itgb7	Integrin β7	NM_013566.2	2	9↑	TNX	[66]
Klf2	Kruppel like factor 2	NM_008452.2	0	13↑	TNX	[66]
Lgals3	Lectin, galactoside-binding soluble 3	NM_010705.2	3	55↑	TNX	[66]
Olfr1265	Olfactory receptor 1265	NM_146343.1	0	8↑	TNX	[66]
Ret	Ret proto-oncogene	NM_001080780.1	2	11↑	TNX	[66]
Ssxb2	Synovial sarcoma, X member B, breakpoint 2	NM_001001450.4	0	12↑	TNX	[66]
Tm9Sf4	Transmembrane 9 superfamily protein member 4	NM_133847.3	4	14↑	TNX	[66]
Traf5	TNF receptor-associated factor 5	NM_011633.1	0	10↑	TNX	[66]
Vps25	Vacuolar protein sorting 25	NM_026776.3	3	12↑	TNX	[66]
Zcchc2	Zinc finger, CCHC domain containing 2	NM_001122675.1	1	9↑	TNX	[66]
Cyp1b1	CYP450 family 1, subfamily B, polypeptide 1	NM_009994.1	4	33↑	TNX, DN-RTE (3↑)	[65,66]
Ccl9	Chemokine (C-C motif) ligand 9	NM_011338.2	1	6↑	DN-RTE	[65]
Clec4d	C-type lectin domain family 4, member d	NM_010819.3	1	4↑	DN-RTE	[65]
Ctsg	Cathepsin G	NM_007800.1	3	6↑	DN-RTE	[65]
Ctsl	Cathepsin L	NM_009984.3	2	4↑	DN-RTE	[65]
Cxcl2	Chemokine (C-X-C motif) ligand 2	NM_009140.2	3	12↑	DN-RTE	[65]
Fn1	Fibronectin 1	NM_010233.1	3	5↑	DN-RTE	[65]
Hp	Haptoglobin	NM_017370.1	2	3↑	DN-RTE	[65]
Lpl	Lipoprotein lipase	NM_008509.2	2	5↑	DN-RTE	[65]
Lyz	Lysozyme	NM_013590.3	1	3↑	DN-RTE	[65]
Mt1	Metallothionein 1	NM_013602.2	2	3↑	DN-RTE	[65]
S100a8	S100 calcium binding protein A8	NM_013650.2	1	4↑	DN-RTE	[65]
S100a9	S100 calcium binding protein A9	NM_009114.2	4	5↑	DN-RTE	[65]
Scin	Scinderin	NM_009132.1	2	11↑	DN-RTE	[65]

Perhaps one of the most consistent phenotypic findings in *Ahr* null animals is a patent ductus venosus (DV).[37,40] The DV, like the ductus arteriosus and foramen ovale, is part of the fetal circulatory system.[41] The DV connects the umbilical/portal vein to the inferior vena cava, allowing oxygen- and nutrient-rich blood filtered by the placenta to bypass the embryonic liver and nourish the developing heart and brain. Shortly after birth, the DV closes and forces blood from the portal vein through the liver sinusoids for hepatic filtration prior to reaching the lungs and heart. In 100% of *Ahr* null animals, the DV remains patent into adulthood.[40] Because of its robust phenotype–genotype correlation, in our laboratory DV closure is used as a marker of developmental AHR signalling. Hypomorphic *Ahr*, *Arnt*, and *Ara9* mice, which express only 1/10th of the normal level of protein, also have a patent DV, highlighting the importance of these AHR signalling pathway members in developmental signalling.[42–44] Because decreased perfusion and necrosis are seen prior to normal DV closure, the patent DV phenotype in *Ahr* null animals may be secondary to the abnormal microvascular perfusion. However, the exact mechanism by which the loss of AHR signalling leads to patent DV is still unknown.

Endogenous ligand

The developmental cue for AHR signalling is still unknown. In support of the existence of an 'endogenous ligand', we offer the fact that activation of the AHR by TCDD rescues the patent DV phenotype of AHR hypomorphs.[43] This observation demonstrates that the developmental response to this toxic ligand mimics developmental AHR signalling. A number of potential endogenous ligands have been suggested. In this regard, indigoids and tryptophan derivatives, which are structurally similar to known xenobiotic ligands, are able to activate AHR signalling.[45,46] Recently, low density lipoprotein (LDL) has also been identified as an activator of AHR signalling.[47] Because LDL has a very different structure from other known AHR ligands, it is possible that LDL carries into the cell a small molecule with more characteristic AHR agonist features.

However, the developmental cue for AHR activation may not be a ligand at all. Alternative activation mechanisms of the AHR, such as intracellular cyclic AMP (cAMP) and fluid shear stress, have been proposed.[47,48] In addition, the AHR may become activated by phosphorylation in response to another cellular cue.[49] There is evidence that is inconsistent with the existence of an endogenous ligand. In this regard, mice carrying both the *Ahr*[b] and *Ahr*[d] alleles, encoding receptors with a 10-fold difference in ligand-binding affinity, display normal DV closure (J. Walisser, unpublished observation). Mouse models have demonstrated that the AHR is required in

different cells for toxicity from those required for the developmental role.[50] Therefore, the mechanism of TCDD toxicity, clearly linked to ligand binding, is unlikely to be secondary to disruption of the normal developmental role of the AHR. A lack of an endogenous ligand for the AHR would suggest that the adaptive and toxic pathways are independent from the developmental pathway. In fact, there is phylogenetic evidence that the ligand-binding functions of the AHR evolved independently from its developmental role.[51]

Evolutionary biology of AHR

While the AHR was discovered because of its role in toxicology, the primary function of mammalian AHR is probably related to normal development. Phylogenetic evidence suggests that the vertebrate AHR arose in biological systems over 450 Ma. Therefore, it is unlikely that the products of modern industrialization, PAHs and dioxins, have provided the selective pressure for the conservation of the AHR throughout evolution.[51,52] Although nearly all vertebrate AHR orthologues identified to date have been shown to bind TCDD, the response to xenobiotic ligands is quite variable.[53,54] The variability in xenobiotic response may have arisen as a means to limit AHR-mediated toxicity while maintaining its key developmental role. Put another way, ligand binding may be a secondary, acquired function of this receptor that arose during vertebrate evolution.

Additional evidence that ligand binding is independent of the developmental role of the AHR comes from data in invertebrate organisms. Invertebrate and vertebrate AHRs share key properties in signal transduction, including heterodimerization with ARNT orthologues and transcriptional activation through DREs.[51,53,55,56] However, invertebrate orthologues of the AHR do not bind classic AHR ligands.[52,54,55] The DNA sequence of the AHR may have been modified during the evolution of vertebrates, to accommodate an increasing need for AHR signalling in vascular development. These same modifications also may have led to the development of ligand binding.

The vertebrate AHR may function in an analogous manner to the invertebrate AHR but in the vasculature. The *Caenorhabditis elegans* AHR orthologue directs neuronal cell fate and oxygen-sensitive aggregation.[57,58] The *Drosophila melanogaster* AHR orthologue is expressed in sensory cells and mutations can lead to increased dendritic branching and overgrowth, antennae transforming into legs, and loss of colour vision.[58] Although there is little evidence that these same roles of invertebrate AHR have been maintained in mammals, the increased density of anastomotic hepatic sinusoids in the *Ahr* null mouse is reminiscent of the increased dendritic branching and overgrowth in *Drosophila*.

AHR in the immune system

AHR-dependent immune function

There is considerable evidence to suggest that AHR signalling plays a role in the function of the immune system. Numerous haematopoietic defects have been described in *Ahr* null mouse models, including altered lymphocyte numbers in the spleen, perinatal extramedullary haematopoiesis in the liver, and enlarged spleens.[33,34] While splenomegaly may be secondary to portal hypertension it may also be a compensation for a haematopoietic defect. In addition to these histological differences, functional studies support the idea that the AHR plays a role in immunity. In this regard, *Ahr* null animals are more susceptible to listeria infection.[59]

Considerable evidence from studies using AHR agonists further supports a role for the AHR in the immune system. Exposure to TCDD leads to profound suppression of both humoral and cellular immune responses and results in increased susceptibility to infection.[60,61] Although TCDD suppresses CD40L-activated B-cell proliferation, T cells are the primary targets of TCDD and mediate inhibition of the antibody response of B cells.[62,63] Thymic involution induced by TCDD is associated with thymocyte loss, thymocyte proliferation arrest and premature emigration of T-cell progenitors.[64–66] In addition, TCDD can prevent prothymocytes in the bone marrow from seeding the thymus.[67] Three independent laboratories have identified an early triple-negative thymocyte population as the targets of TCDD-induced thymocyte emigration.[64–66] Exposure to AHR agonists also affects functional immunity. For example, TCDD causes increased inflammation and inhibits the CD8$^+$ T-cell response to influenza infection.[68,69] Other model systems shown to be affected by AHR agonists include experimental autoimmune encephalitis (EAE), graft-versus-host disease (GVHD), and mouse models of allergy and transplant tolerance.[70–75]

The AHR and Tregs

Recently a role for AHR signalling in regulatory T cells (Tregs) has been reported by at least four independent laboratories. By suppressing effector cell proliferation and cytokine secretion, Tregs have been shown to reduce autoimmune and allergic disease, limit the immune response in infectious disease, and inhibit antitumour immune responses.[76] There is evidence that a subset of Tregs develop in the thymus, known as natural or innate Tregs.[77] Tregs also develop in the periphery during an immune response and are referred to as adaptive Tregs. These two populations of Tregs probably differ in their antigen specificity, development, and mechanism of immune regulation.[78,79] In one report, exposure to TCDD increased the proliferation of Tregs and suppressed EAE.[70] In another report, TCDD exposure generated Tregs and prevented GVHD.[72] In a third model, activation of the AHR also induced Tregs and improved graft survival.[75] In keeping with a role for the AHR in Treg function, it has been observed that naïve T cells isolated from *Ahr* null animals are inefficient at generating Tregs *in vitro*.[80]

The mechanism by which AHR signalling might promote Treg differentiation remains largely uncharacterized. There are many ways to define Tregs. However, there is not yet a clear way to differentiate natural and adaptive Tregs.[78] Some laboratories define Tregs by their *in vitro* suppressive activity or expression of the cell surface markers CD25 and CD62L.[72,81] Other laboratories use the expression of forkhead box P3 (FoxP3), a transcription factor thought to play a central role in Treg activity.[70,80] Exposure to TCDD causes a reduction in CD62L expression in T cells.[82] There is evidence that the AHR directly regulates the expression of FoxP3, and AHR activation leads to an increase in Tregs in at least two model systems.[70,75,80] However, a decrease in FoxP3$^+$ cells upon activation of the AHR was seen in a GVHD model.[72] This inconsistency in the effect of AHR signalling on FoxP3$^+$ cells may be explained by the dose of TCDD or the immune response model used in the experiments. The variable effect on FoxP3 expression also may suggest that AHR signalling plays different roles in natural and adaptive Tregs and that the type of Tregs involved in EAE, allograft-tolerance and GVHD models differs.

The AHR may affect Treg differentiation through at least two other mechanisms. First, AHR signalling may influence Treg development by augmenting transforming growth factor (TGF)-β signalling. A number of independent laboratories using a variety of ligands and experimental systems have identified an interplay between the AHR and TGF-β signalling pathways.[83–86] Cross-talk between AHR and TGF-β signalling also has been observed during Treg differentiation. For example, a 13-fold increase in TGF-β3 RNA was found in Tregs exposed to TCDD.[72] Furthermore, in tissue culture, TGF-β mimics the effects of TCDD on Tregs, and inhibition of TGF-β signalling also inhibits TCDD-induced Treg activity.[70] It has been suggested that the Treg populations have different requirements for TGF-β.[78] In fact, there are fewer peripheral Tregs, but normal numbers of thymus-derived Tregs, in *Tgfbr* null mice.[87] These data also provide evidence that the AHR may play different roles in adaptive and natural Tregs.

Another potential mechanism by which the AHR may affect Treg activity involves dendritic cells (DCs). DC antigen presentation plays a central role in converting naïve T cells into Tregs in the periphery (adaptive Tregs).[88,89] Cytokines are crucial to T-cell activation, and without the appropriate milieu, DCs can induce clonal deletion, anergy or tolerogenic regulatory T cells.[90,91] A

model exists whereby mature DCs activate Tregs, and these Tregs go on to limit their own expansion by blocking splenic DCs from maturing.[89] Exposure to TCDD reduces the number of splenic DCs.[92] The AHR agonist VAF347 promoted long-term graft acceptance in an islet cell transplant model and reduced the response in an allergy model.[74,75] In this study, alterations in DC expression of interleukin (IL)-6, IL-10 and TGF-β were proposed as a potential mechanism of the AHR-mediated effect. In fact, the authors demonstrated that graft rejection was prevented by transfer of AHR agonist-treated DCs. Taken together, these findings suggest that AHR signalling may affect Treg differentiation by modulating expression of Treg markers from within Tregs or through DCs.

AHR-mediated inflammation

The AHR has been reported recently to play a role in the development of T helper 17 (Th17) cells, a new subset of CD4^{+} T cells thought to play a major role in autoimmunity and clearance of infectious agents. It has been observed that injecting healthy mice with Th17 cells from animals with EAE causes autoimmunity in the recipients.[93] In addition, it has been shown that adequate Th17 cell function inhibits systemic infection with gastrointestinal pathogens.[93,94] Th17 cells are characterized by their secretion of the proinflammatory cytokines IL-17 and IL-22. The ligand-activated AHR regulates expression of these cytokines in tissue culture.[71] A role for the AHR in the regulation of Th17 cells is supported further by the observation that the absolute number of Th17 cells is reduced in *Ahr* null mice upon induction of EAE.[71,80]

Because Th17 cells promote the immune response and Tregs are known to decrease immune reactivity, a model has emerged suggesting that the Treg/Th17 cell balance distinguishes an effective immune response and self-antigen tolerance from chronic infection or autoimmunity. Preliminary evidence from multiple laboratories has suggested that the AHR modifies the Treg/Th17 cell balance through modifying the cytokine milleu. The mechanism at work may be related to the fact that TGF-β induces Treg differentiation, while the presence of IL-6 leads to TGF-β-dependent Th17 cell production.[76,93] As described above, the AHR has been shown to modulate cytokine signalling. Further evidence for this biology is provided by unpublished observations from our own laboratory, where we observed that *Helicobacter* infection results in rectal prolapse in *Ahr* null animals, in marked contrast to wild-type littermates (E. Stevens, unpublished observation). It is possible that an alteration in the Treg/Th17 balance in *Ahr* null animals may play a role in the severity of this gastrointestinal infection.

As a transcription factor, AHR probably modulates T-cell development at the transcriptional level. In addition to subtype-specific transcriptional changes described above, the up-regulation of CD11a is blocked in activated T cells treated with TCDD, which may impede T cells from reaching the source of antigen.[82] As chemokine receptor transcripts are up-regulated, T cells disappear from the spleen, suggesting homing to other tissues.[72] It has also been demonstrated that TCDD up-regulates Kruppel like factor 2 (*Klf2*), which is implicated in the homing of T cells and the prevention of inflammatory chemokine receptor expression.[66,95] In summary, the published data to date are in agreement that AHR-mediated transcriptional changes can affect T-cell activation, but the mechanism is still largely unknown.

Exposure to TCDD can lead to inflammation and also to increased secretion of inflammatory cytokines involved in innate immunity.[28] In mice, TCDD exposure is associated with decreased survival, neutrophilia, and elevated interferon (IFN)-γ levels in the lungs following influenza virus infection.[68] Given that AHR in haematopoietic cells is not required for this phenotype, it may be that activation within the lung parenchyma changes the immune response to infection.[68,96] One interpretation of this data is that neutrophils are a secondary response to AHR-mediated transcriptional changes within the lung parenchymal target cell. This model may be similar to what is occurring in TCDD-induced hepatotoxicity. In this system, conditional AHR mouse models have been used to demonstrate that TCDD causes primary transcriptional effects within hepatocytes and secondary effects are mediated by inflammatory cells that exacerbate the hepatotoxicity.[97] These experiments emphasize the importance of determining the primary effects of AHR signalling in order to elucidate the mechanism by which AHR signalling affects the immune response.

Considerations for future experiments

Primary and secondary effects in AHR biology

We propose a model in which all the upstream signalling steps in the three AHR biological pathways are similar. To support this idea, we offer the fact that both TCDD-induced toxicity and vascular development are dependent on most, if not all, of the same signalling molecules required for the adaptive response.[42,44] Given the importance of enhancer recognition of the AHR:ARNT heterodimer, it would follow that transcriptional changes are the primary mechanism leading to both developmental and toxic end-points. In support of this idea, AHR mutants deficient in DNA-binding or nuclear localization activity are also resistant to TCDD-induced toxicity and display patent DV.[98,99] In our view, these experiments suggest that elucidating the specific transcriptional targets of AHR underlying each distinct biological end-point is critical to defining the mechanism.

One key point is that, while the signalling mechanism of gene transcription is central to all AHR biology, which genes are targeted depends largely on the cell type being studied. Data demonstrating that the AHR is required in different cell types for hepatotoxicity and vascular development are in agreement with the idea that the AHR mediates various roles in a cell type-dependent fashion.[50] Therefore, identifying the target cell(s) is an important first step in the identification of key gene targets mediating such variable end-points as hepatoxicity, vascular remodelling, and immune suppression in AHR biology.

Another key point to consider when studying the mechanism by which the AHR mediates such diverse biology is the distinction between primary and downstream gene targets. While DRE-driven, AHR-mediated transcription has been well studied, DRE-independent gene transcription has also been reported to occur. These DRE-independent targets may represent promoters bound by the AHR at enhancer sequences distinct from DREs or they may be secondary targets.[100] Using dose response and timing of transcriptional changes, the primary genetic targets of AHR activation in the liver have been separated from the downstream transcriptional changes during TCDD-induced hepatoxicity.[101,102] Although many AHR target genes have been identified, their position in the signalling sequence and the mechanism by which they mediate AHR biology still must be elucidated (Table 1). In conclusion, identifying the primary transcriptional changes mediated by the AHR in target cells holds promise for elucidation of the role of AHR in the immune system.

AHR pharmacology

The well-characterized pharmacology of the AHR may prove to be a powerful tool with which to unravel the role of this protein in immunology. The most studied AHR agonist, TCDD, binds AHR with high affinity.[103,104] Because its four chlorine residues prevent access to the active sites of metabolic enzymes, TCDD is poorly metabolized.[103] As a relatively pure, high-affinity agonist, TCDD can be used at low doses and thus would be predicted to have fewer off-target effects than non-halogenated agonists. For example, indirubin is a potent AHR agonist, but it also binds to and inhibits cyclin-dependent kinases and c-Src kinase.[105] Benzo-*a*-pyrene activates the AHR and is metabolized to epoxides and quinones via the adaptive pathway.[106] Epoxide intermediates are known to be highly cytotoxic through alkylation of DNA and other cellular macromolecules.[106] Quinones can generate reactive oxygen species (ROS), which in turn can influence gene expression through a variety of mechanisms, including activation of the transcription factor nuclear erythroid 2-related factor (Nrf2).[106,107] The bottom line is that studies using highly potent and specific agonists, such as TCDD, provide the most direct route to elucidate the signalling steps by which the AHR influences the immune system.

Another important pharmacological consideration in the study of AHR signalling is the length of activation. Unlike TCDD, many AHR agonists, including benzo-a-pyrene (BaP), indirubin and 6-formylindolo(3,2-*b*) carbazole (FICZ), are rapidly cleared, leading to only short-term activation.[103] These agonists cause substantial up-regulation of AHR battery genes, but only within hours of treatment.[103,108] In comparison, TCDD causes long-term stimulation of AHR that can be measured days after administration.[109–111] Differences in the length of AHR stimulation may lead to differences in AHR-mediated biology. In fact, data from experiments using FICZ and TCDD led to different conclusions about AHR signalling in T cells during EAE.[70] It is clear that the choice of agonist is an important consideration for the design and interpretation of future studies (Table 2).

Use of *Ahr* mutant animals

The use of *Ahr* null animals complicates AHR pharmacology. There are two reasons why the use of *Ahr* null animals can lead to misinterpretation of results. First, these mice have a patent DV and other abnormalities described above. It can, therefore, be difficult to isolate phenotypic effects directly caused by the loss of the AHR and those downstream of abnormal vasculature. Secondly, *Ahr* null animals should not be used to study ligands that may be modified by metabolic enzymes. Bioactivation may be required for the phenotypic effect. The use of *Ahr*

Table 2. Aryl hydrocarbon receptor (AHR) ligands

Ligand	EC_{50} (mol/kg)	Metabolized?	References
HAH (TCDD)	10^{-12}	No	116,117
PAH (BaP)	10^{-5}–10^{-6}	Yes	117,118
PCB (pentaCB)	10^{-7}	Yes	104
FICZ	10^{-10}–10^{-12}	Yes	109,119
Indirubin	10^{-8}	Yes	45,120
Lipoxin-4a	10^{-9}	Yes	121
Bilirubin	10^{-6}	Yes	122
ITE	10^{-9}	?	123,124
ICZ	10^{-8}–10^{-10}	Yes	116,125

This table contains some of the best studied AHR ligands. The EC_{50} is the dose of the ligand that leads to 50% of the maximal cytochrome P450 gene induction. These are estimates and are dependent on many factors, including cell type and AHR allele. Some AHR ligands are metabolized enzymatically and are short-lived. The EC_{50}s of these ligands are sensitive to the timing of induction.
FICZ, 6-formylindolo(3,2-*b*)carbazole; ICZ, indolo(3,2-*b*)carbazole; ITE, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; TCDD, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin.

null animals in these situations does not allow the conclusion that the phenotype is the direct result of an AHR transcriptional response, as loss of the AHR impairs metabolic enzyme induction. To test the requirement for the AHR in the pharmacology of any compound, it is imperative to create mice with the *Ahr* deleted specifically in a target cell subset, to avoid patent DV and its resulting pathologies. Such a mouse model can be created with bone marrow chimeras or with the conditional *Ahr^fx* allele and cre recombinase mediated LoxP site recombination (Cre–Lox) technology.

Conclusion

Much has been elucidated about AHR biology in the last 30 years. It is clear that this receptor is not simply a transcription factor developed to respond to toxicants, but probably plays a central role in vascular biology. Activation of this receptor has long been known to cause immunosuppression and thymic involution. Recent data have implicated this receptor in T-cell differentiation and DC function. There is little dispute that T-cell lineage specificity is largely determined by transcription factors and that gene transcription plays large roles in immune responses in other haematopoietic lineages. As a transcription factor, the AHR probably modulates immune reactions and also causes immunotoxicity through transcriptional changes. It seems likely that a role for this receptor in the function of the immune system will be defined.

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