

ASSOCIATE EDITOR: PAUL A. INSEL

Aryl Hydrocarbon Receptor Control of Adaptive Immunity

Francisco J. Quintana and David H. Sherr

Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts (F.J.Q.); and Department of Environmental Health, Boston University School of Public Health, Massachusetts (D.H.S.)

Abstract.....	1148
I. Introduction.....	1148
II. Aryl Hydrocarbon Receptor Signaling Pathways.....	1149
A. Genomic Pathways of Aryl Hydrocarbon Receptor Signaling.....	1149
B. Nongenomic Pathways of Aryl Hydrocarbon Receptor Signaling.....	1150
III. The Aryl Hydrocarbon Receptor in Immunity and Autoimmunity.....	1150
A. Foxp3 ⁺ Regulatory T Cells.....	1150
B. Type 1 Regulatory T Cells.....	1151
C. Th17 Cells.....	1152
D. Effects of Aryl Hydrocarbon Receptor on Antigen Presenting Cells.....	1153
IV. Endogenous Aryl Hydrocarbon Receptor Ligands and Amplification of Aryl Hydrocarbon Receptor Activation with Positive Feedback Loops.....	1153
A. Indirect Evidence of Endogenous Aryl Hydrocarbon Receptor Ligands.....	1154
B. Heme-Derived Molecules.....	1155
C. Arachidonic Acid Metabolites.....	1155
D. Tryptophan Metabolites.....	1155
E. Aryl Hydrocarbon Receptor Feedback Loops.....	1156
V. Aryl Hydrocarbon Receptor as a Therapeutic Target.....	1157
VI. Conclusions.....	1158
Acknowledgments.....	1158
References.....	1159

Abstract—The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that belongs to the family of basic helix-loop-helix transcription factors. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants, the activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the immune response. Thus,

the AhR provides a molecular pathway through which environmental factors modulate the immune response in health and disease. In this review, we discuss the role of AhR in the regulation of the immune response, the source and chemical nature of AhR ligands, factors controlling production and degradation of AhR ligands, and the potential to target the AhR for therapeutic immunomodulation.

Research in the Quintana laboratory was supported by the National Institutes of Health National Institute of Allergy and Infectious Diseases [Grants AI075285 and AI093903]; the National Multiple Sclerosis Society; and the Juvenile Diabetes Research Foundation. Research in the Sherr laboratory was supported by the National Institutes of Health National Institute of Environmental Health Sciences [Grants P01ES11624 and P42ES007381]; and the Art beCAUSE Breast Cancer Foundation.

Address correspondence to: Francisco J. Quintana, Center for Neurologic Diseases, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115. E-mail: fquintana@rics.bwh.harvard.edu or David H. Sherr, Boston University School of Public Health, 72 East Concord St., R-408, Boston, MA 02118. E-mail: dsherr@bu.edu
dx.doi.org/10.1124/pr.113.007823

I. Introduction

Immune-mediated disorders result from complex interactions between genetic and environmental factors. Large-scale collaborative studies during the last decade have resulted in significant advances in the identification of genetic variants that influence the development of immune-mediated disorders (Gregersen and Olsson, 2009). Many of those genetic variants impact specific immune pathways, thereby suggesting mechanistic hypotheses about their role in health and disease.

Although environmental factors also are known to play a role in the onset and progression of autoimmune

disorders, systematic and comprehensive studies evaluating their impact on immune pathways are limited. Therefore, the molecular mechanisms mediating the effects of the environment on the immune response are not completely understood.

Several environmental factors can influence immune function. The commensal flora has significant effects in the control of systemic inflammation and the development of autoimmune disorders in animal models (Lathrop et al., 2011). Indeed, alterations in the composition of the commensal flora have been described in immune-mediated disorders (Kranich et al., 2011). Moreover, infections influence the immune response directed against self-antigens and have been linked to the worsening or the amelioration of immune-mediated disorders. Indeed, immunologic outcomes appear to be linked to the nature of the infectious agents (Yazdanbakhsh et al., 2002; Bach, 2005; Christen and von Herrath, 2005; Kamradt et al., 2005; Kivity et al., 2009; Macia et al., 2012). Diet also affects the immune response and the development of inflammation (Mora et al., 2008; Veldhoen and Brucklacher-Waldert, 2012). Finally, additional factors such as environmental pollutants and ultraviolet light have significant effects on experimental models of autoimmunity and have been linked to human inflammatory disorders (Rook, 2011; Halliday et al., 2012). Although the environment clearly plays a role in the control of immune function, the identity of most of the chemical compounds or perturbations mediating the effects of the environment on the immune response is unknown, making it difficult to identify the molecular mechanisms by which these factors affect immunity.

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates several immune processes. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants, several dietary compounds and products of commensal flora activate AhR signaling at physiologically relevant doses and with significant potency. Thus, the AhR provides a molecular pathway through which environmental factors modulate the immune response and, consequently, the development of immune-mediated disorders. In this review, we will discuss the sources and chemical nature of AhR ligands and the role of AhR on the regulation of the immune response. We suggest that there are multiple “natural” AhR ligands that effect different immunologic outcomes and that production of these ligands is controlled by a complex set of positive and negative feedback loops.

II. Aryl Hydrocarbon Receptor Signaling Pathways

AhR has a promiscuous binding site that can interact with a broad array of synthetic and natural ligands (Nguyen and Bradfield, 2008). Although “ligand-independent” AhR activation has been described in some experimental setups (Oesch-Bartlomowicz et al., 2005), the physiologic relevance of AhR activation in the absence of a ligand is uncertain. Most of the effects of AhR on the immune response apparently involve its activation by one or more ligands. Indeed, the AhR was initially identified as a receptor for dioxins, and much of our understanding on the biology of AhR results from experiments performed using 2,3,7,8-tetracholrodibenzo-*p*-dioxin (TCDD). These studies led to the identification of genomic and nongenomic pathways of AhR signaling.

A. Genomic Pathways of Aryl Hydrocarbon Receptor Signaling

The inactive, cytosolic AhR is part of a protein complex that includes the 90-kDa heat shock protein (HSP90), the c-SRC protein kinase, and the AhR-interacting protein Ara9 (Fig. 1). Ligand binding triggers conformational changes that expose a protein kinase C target site, phosphorylation of which triggers the translocation of the AhR to the nucleus and the dissociation of the AhR/HSP90/c-SRC complex. Once in the nucleus, the AhR establishes protein-protein interactions with the AhR nuclear translocator and with additional coactivators and transcription factors, such as Nco1 and Nco3, Ep300 (p300), and the general transcription factor IIB (Gt ζ 2b) (McIntosh et al., 2010). These protein complexes interact with specific sequences in target genes to control their transcriptional activity. In addition, the AhR interacts with other transcription factors with well-characterized functions in the immune system and in other organ systems (e.g., signal transducer and activator of transcriptions, the retinoic acid receptor, c-maf, the estrogen receptor, E2F, Rb, and nuclear factor- κ B) (Hankinson, 2005). Although AhR complexes clearly interact with the canonical AhR binding site (5'-TNGCGTG-3'), recent data indicate non-canonical recognition sequences as well (Huang and Elferink, 2012), suggesting the likelihood that the AhR controls transcription of significantly more genes than previously appreciated. It is striking that several AhR protein interactions are only triggered by specific AhR ligands (Boronat et al., 2007; Zhang et al., 2008; Murray et al., 2010), suggesting that some transcriptional partners of AhR are recruited in a ligand-specific manner.

ABBREVIATIONS: AhR, aryl hydrocarbon receptor; APCs, antigen presenting cells; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; FICZ, 6-formylindolo[3,2-*b*]carbazole; HETEs, 4-hydroxyeicosatrienoic acids; HSP90, 90-kDa heat shock protein; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; ITE, 2-(1*H*-indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester; iTregs, induced FoxP3⁺ Tregs generated in the periphery; NPs, nanoparticles; nTregs, natural FoxP3⁺ Tregs generated in the thymus; PG, prostaglandin; RA, retinoic acid; TCDD, 2,3,7,8-tetracholrodibenzo-*p*-dioxin; TLR, Toll-like receptor; Tr1 cells, Type 1 regulatory T cells; Tregs, regulatory T cells.

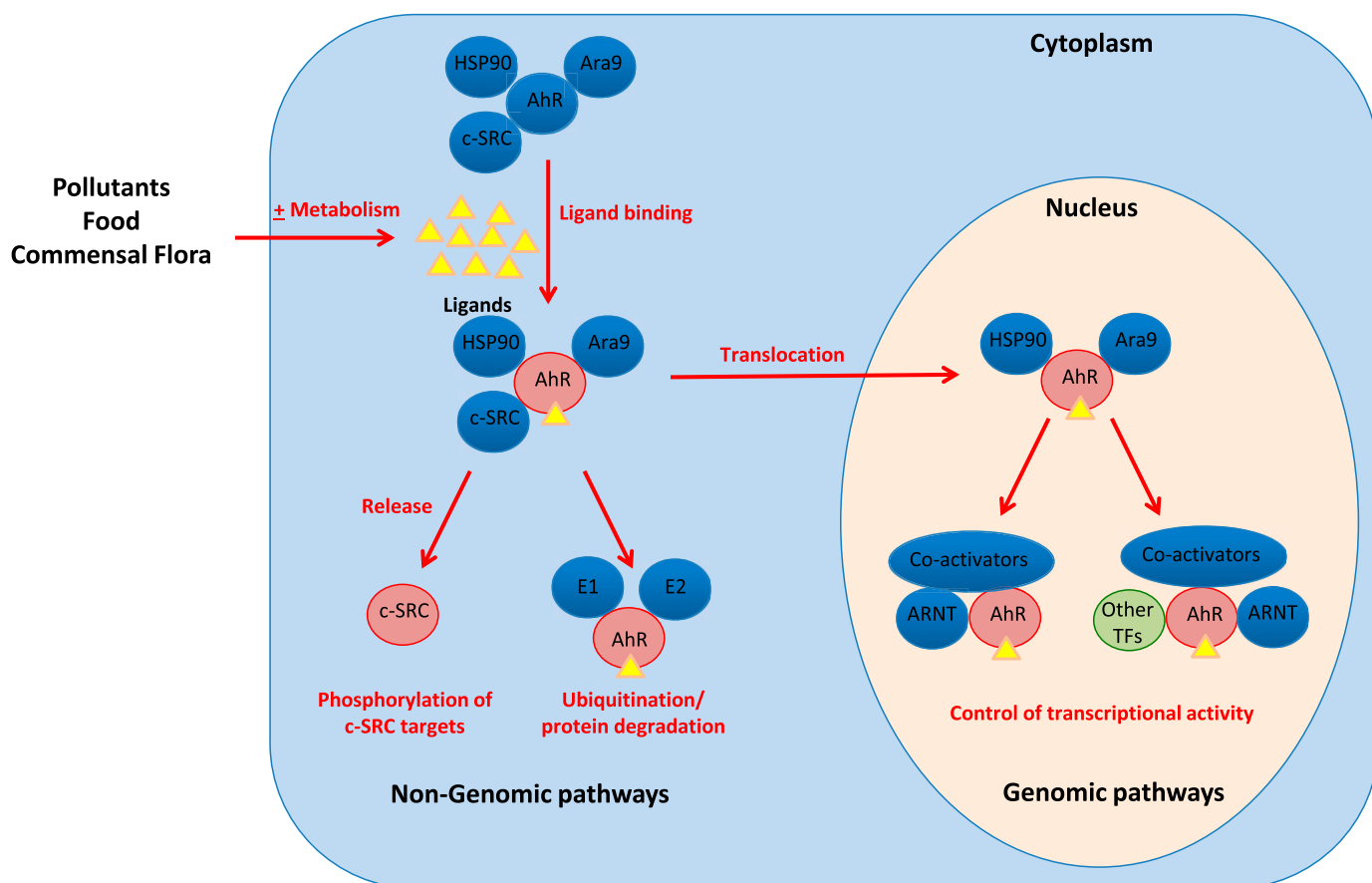


Fig. 1. AhR signaling pathways. AhR ligands, or precursors to AhR ligands, are derived from industrial waste or by-products (pollutants), cruciferous plants (diet), or commensal flora. Several endogenous ligands also are proposed. Upon interaction with its ligands, the AhR undergoes conformational changes that result in its translocation to the nucleus and the dissociation of the AhR/HSP90/c-SRC complex. Once in the nucleus, the AhR establishes protein-protein interactions with the AhR nuclear translocator (ARNT) and with additional coactivators and transcription factors (TF) to control the transcriptional activity of target genes. In addition, the conformational changes induced by ligand binding result in the release of active c-SRC, which then targets multiple cellular targets. AhR is also a ligand-dependent E3 ubiquitin ligase that directs the ubiquitination and degradation of target proteins by the proteasome.

B. Nongenomic Pathways of Aryl Hydrocarbon Receptor Signaling

In addition to its direct transcriptional regulation of target genes, the AhR also has been shown to control cellular processes through nongenomic signaling pathways. One of these pathways results from the release of c-SRC from the AhR/HSP90/c-SRC complex, enabling c-SRC to then target multiple cellular targets (Matsumura, 2009). In addition, the AhR has been reported to control the proteasome-dependent degradation of specific transcription factors. The proteasome system catalyzes the degradation of ubiquitinated proteins, an important process for cellular homeostasis (Hershko and Ciechanover, 1998). Ubiquitin is covalently attached to target proteins through a sequence of reactions catalyzed by ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3). E3 ubiquitin ligases control the specificity of the ubiquitination reaction. Ohtake et al. (2007) reported that the AhR is a ligand-dependent E3 ubiquitin ligase involved in the degradation of AhR-interacting proteins such as the estrogen receptor. This surprising finding

suggests that, by means of its E3 ligase activity, the AhR might promote the degradation of specific interaction partners. However, the relevance of this pathway for the effects of AhR on the immune response is still unknown.

III. The Aryl Hydrocarbon Receptor in Immunity and Autoimmunity

The AhR plays an important role in the control of the adaptive immune response. In particular, we and others have found that the AhR controls the differentiation and activity of specific T-cell subpopulations. The AhR influences adaptive immune responses through its effects on both T cells and antigen presenting cells (APCs). In this section we discuss the direct (T-cell-mediated) and indirect (APC-mediated) effects of the AhR on different T-cell development and function.

A. *Foxp3*⁺ Regulatory T Cells

Several specialized T-cell populations control the immune response. One of these populations is composed of CD4⁺ regulatory T cells (Tregs) that express

the transcription factor FoxP3, which is needed for their differentiation and function (Sakaguchi et al., 2010). FoxP3⁺ Tregs play a central role in the control of immune reactivity to self and non-self antigens, as shown by the inflammatory conditions associated with mutant FoxP3 in humans (Sakaguchi et al., 2010) and in mice (Fontenot et al., 2003; Hori et al., 2003; Wan and Flavell, 2007). On the basis of their origin, FoxP3⁺ Tregs can be classified as natural FoxP3⁺ Tregs (nTregs; generated in the thymus) or induced FoxP3⁺ Tregs (iTregs; generated in the periphery). Although both nTregs and iTregs are characterized by FoxP3 expression, they differ in their stability, some of the pathways controlling their generation and, probably, their physiologic functions. AhR has been shown to play important roles in the development and function of both nTregs and iTregs.

The immune system of zebrafish shares many features with its mammalian counterpart. We reported that the zebrafish immune system can mediate self-directed inflammation and tissue destruction (Quintana et al., 2010a). This potential self-reactivity is controlled by functional FoxP3⁺ Tregs, suggesting that the potential for autoimmunity co-evolved with FoxP3-based mechanisms of immunoregulation. However, although this has not been formally probed, the FoxP3⁺ Treg compartment has been proposed to include only FoxP3⁺ nTregs, because iTregs have been associated with the placenta and the need to establish immune-tolerance to the developing fetus. Using zebrafish, we identified the AhR as a potential regulator of FoxP3 expression and Treg differentiation (Quintana et al., 2010a). Indeed, AhR activation with TCDD leads to a significant suppression of inflammation and interleukin (IL)-17 expression in zebrafish (Quintana et al., 2010a).

We and others then performed extensive studies to characterize the effects of the AhR in FoxP3⁺-positive cells. The AhR both directly induces the expression of FoxP3 (Quintana et al., 2008) and controls its epigenetic status, making it more accessible to the transcription machinery (Singh et al., 2011b). In addition to its direct effects on the expression of FoxP3, AhR activation also increases the expression of SMAD1 in human Tregs, which, through its activity on a conserved enhancer, stabilizes FoxP3 expression (Gandhi et al., 2010). Moreover, AhR limits STAT1 activation, which is known to inhibit the differentiation of FoxP3⁺ Tregs (Kimura et al., 2008; Quintana et al., 2010b).

FoxP3 promotes the differentiation of Tregs by both promoting the expression of target genes and suppressing the expression of a set of genes associated with effector T-cell responses (Josefowicz et al., 2012). The inhibitory effects of FoxP3 on the expression of target genes is mediated by its interaction with Eos (Pan et al., 2009; Bettini et al., 2012; Fu et al., 2012), a transcription factor belonging to a family of proteins that also includes Ikaros (the founding member of this protein family), Pegasus, Helios, and Aiolos. In addition to Eos,

other members of the Ikaros family of transcription factors, such as Helios, are expressed by Foxp3⁺ Tregs (Thornton et al., 2010; Gottschalk et al., 2012). We found that the AhR controls the expression of Aiolos in both human and mouse FoxP3⁺ Tregs (Gandhi et al., 2010; Quintana et al., 2012). Indeed, we demonstrated that FoxP3⁺ interacts with the C-terminal zinc fingers in Aiolos to form a protein complex that silences IL-2 expression in FoxP3⁺ Tregs. Thus, the AhR participates in both the induction of Treg-specific genes, such as FoxP3, and the inhibition of genes associated with effector T-cell function, such as IL-2.

In addition to the FoxP3⁺ iTregs and nTregs, identified in part by their origin, different Treg subpopulations can be identified by their expression of specific transcriptional modules that likely control development or function of specific effector T-cell subpopulations. We recently found that a subpopulation of FoxP3⁺ T cells, characterized by the expression of high levels of membrane T-cell immunoreceptor with Ig and ITIM domains, expresses high AhR levels (unpublished data). This FoxP3⁺ Treg population is specialized in its suppression of effector T-cell responses through a mechanism that involves the expression of the suppressive molecule Fgl2 under the control of the transcription factor CCAAT/enhancer-binding protein α . Although the relationship between the AhR and the CEBP α /Fgl2 axis is still unclear, these data suggest that the AhR is disproportionately associated with specific subpopulations of regulatory T cells.

B. Type 1 Regulatory T Cells

FoxP3⁻ IL-10⁺ CD4⁺ Tr1 cells play an important role in the regulation of tissue inflammation, particularly at mucosal sites. Tr1 cells were initially described to be induced in response to repeated stimulation in the presence of IL-10 (Groux et al., 1997) or in response to activation in the presence of rapamycin and vitamin D (Gregori et al., 2011). Although additional *in vitro* and *in vivo* experimental protocols promote differentiation of IL-10⁺ CD4⁺ T cells with regulatory function, it was later shown that IL-27 signaling consistently promotes the differentiation of Tr1 cells *in vitro* (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007).

The concept that AhR activation could suppress immune responses was first suggested by Kerkvliet et al. (1990). As early as 1990, Kerkvliet et al. demonstrated that TCDD inhibited allo-specific cytotoxic T-cell responses in an AhR-dependent manner. Subsequent studies demonstrated that this immunosuppression was at least in part attributable to TCDD-mediated induction of FoxP3⁻ Tregs (Kerkvliet et al., 2002; Marshall et al., 2008).

We found that AhR plays several roles during the differentiation of Tr1 cells *in vitro* and *in vivo* (Apetoh et al., 2010; Gandhi et al., 2010; Wu et al., 2011). AhR activation with different ligands boosts the differentiation

of Tr1 cells in vitro (Apetoh et al., 2010; Gandhi et al., 2010). Conversely, *Ahr* deficiency results in a reduced differentiation of Tr1 cells in vivo in response to treatment with anti-CD3 (Apetoh et al., 2010; Wu et al., 2011). Taken together, these data suggest that the AhR controls the differentiation of Tr1 cells.

A comprehensive transcriptional profile of Tr1 cells is still missing. However, several transcription factors important for the control of Tr1 cell differentiation and activity have been identified. Our data suggest that the AhR is functionally linked to several of the transcription factors reported to participate in the differentiation of Tr1 cells. Whole genome chromatin immunoprecipitation studies detected a significant binding of STAT3 to the AhR promoter, suggesting that STAT3 controls AhR expression (Durant et al., 2010). Therefore, some of the STAT3-dependent effects on Tr1 cell differentiation might be mediated by IL-27.

c-Maf transactivates the *il10* promoter in T cells (Xu et al., 2009). Indeed c-Maf is associated with the production of IL-10 in several cell types and has therefore been proposed to be a universal transcription factor controlling IL-10 production (Saraiva and O'Garra, 2010). We found that the AhR physically interacts with c-maf to cooperatively transactivate the human and mouse *il10* promoter (Apetoh et al., 2010). Interestingly, the AhR can interact with the *il10* promoter in the absence of exogenous IL-27, suggesting that the AhR marks regulatory regions in the *il10* promoter to facilitate the recruitment of the transcriptional complexes needed for its rapid activation in Tr1 cells. Indeed, a similar mechanism was recently described for the rapid induction of certain genes in DCs. Of note, AhR deficiency also impairs IL-10 production in macrophages (Kimura et al., 2009), suggesting that the formation of a c-maf/AhR complex might participate in the role of c-maf as a universal regulator of *il10* expression.

We recently showed that the AhR, in cooperation with STAT3, controls the expression of the transcription factor Aiolos, which is expressed by IL-27-induced Tr1 cells (Quintana et al., 2012). Although the effects of Aiolos on IL-10 expression have not been investigated, Ikaros, which belongs to the family of transcription factors that includes Aiolos, is known to transactivate the *il10* promoter. Moreover, STAT3 has been shown to interact with the *il10* promoter in T cells (Chaudhry et al., 2011). Thus, it is possible that the AhR controls the transcriptional activity of the *il10* promoter through its interactions with additional transcription factors other than c-maf, such as Aiolos and STAT-3. Moreover, the expression of the AhR, c-Maf, and Aiolos is controlled by STAT-3 in T cells, and, as we previously mentioned, AhR interacts with and modulates the activity of STAT proteins (Kimura et al., 2008; Veldhoen et al., 2009; Quintana et al., 2010b). Thus, it is possible that AhR participates in a feedback loop that regulates STAT3 activation during Tr1 cell differentiation.

Finally, IL-21 is a growth factor that works in an autocrine manner to boost IL-10 production and to expand and stabilize Tr1 cells. IL-21 production is also controlled by c-maf in Tr1 cells. Indeed, we found that the AhR/c-maf complex binds and transactivates the *il21* promoter, controlling IL-21 expression by Tr1 cells. Taken together, these data indicate that the AhR acts at several levels of Tr1 cell differentiation.

C. *Th17* Cells

We (Quintana et al., 2008) and others (Kimura et al., 2008; Veldhoen et al., 2008) reported that AhR plays an important role during the differentiation of Th17 cells. Activation of the AhR in vitro promotes the generation of IL-17⁺ T cells and increases production of IL-21 and IL-22. Indeed, Veldhoen et al. (2008, 2009) demonstrated that AhR deficiency abrogates IL-22 production by Th17 cells. However, and unlike the role of the AhR in the control of *il10* expression in Tr1 cells, AhR does not directly control the expression of *il17* (Ciofani et al., 2012). Instead, AhR regulates the inhibitory effects of IL-2 on the differentiation of Th17 cells.

IL-2 interferes with the differentiation of Th17 cells, particularly during the early stages of their differentiation (Laurence et al., 2007; Liao et al., 2011; Yang et al., 2011). This inhibition results from the competition between STAT5 and STAT3 for DNA sites that regulate the expression of the Th17 transcriptional program (Chen et al., 2011; Pandiyan et al., 2011). Thus, it has been postulated that the differentiation of Th17 cells in vivo requires FoxP3⁺ Tregs that uptake IL-2 and alleviate its inhibitory effects on Th17 cells. IL-2 activates STAT5 in T cells and, as we already mentioned, AhR interacts with STAT proteins to modulate their activity (Kimura et al., 2008). Accordingly, Veldhoen et al. (2009) found that the AhR limits the activation of STAT5 in Th17 cells, thereby interfering with the inhibitory signals provided by IL-2 on Th17 cell differentiation.

In addition to the effects of AhR on STAT5 signaling, we recently found a transcriptional module by which the AhR limits the production of IL-2 during Th17 cell differentiation. We found that the AhR cooperates with STAT3 to induce the expression of the epigenetic modifier Aiolos in Th17 cells. Aiolos modifies the epigenetic status of the *il2* promoter, limiting its accessibility to transcription factors and effectively silencing IL-2 expression. This cell-autonomous inhibition of IL-2 production alleviates the inhibitory effects of IL-2 on the early stages of Th17 cell differentiation.

The inhibitory effects of IL-2 are only detected during the early stages of Th17 cell differentiation. Indeed, AhR expression is highest in Th17 cells that have not fully developed an inflammatory phenotype (Lee et al., 2012a,b). These cells are characterized by the expression of IL-10 and a limited capacity to mediate inflammation

in certain experimental setups (McGeachy et al., 2007). Taken together, these data suggest that the AhR might play different roles at different stages of Th17 cell differentiation. During early stages, AhR might limit the inhibitory effects of IL-2 and promote IL-10 production, whereas in late stages it might contribute to the expression of IL-21 and IL-22.

D. Effects of Aryl Hydrocarbon Receptor on Antigen Presenting Cells

Dendritic cells (DCs) play a central role in the control of T-cell activation and polarization *in vivo*. In the context of organ-specific autoimmunity, for example, DCs control the balance between effector and regulatory T cells (Lewis and Reizis, 2012; Satpathy et al., 2012), and dysfunctional DCs have been described in human autoimmune diseases (Comabella et al., 2010). The AhR can control the differentiation and function of DCs *in vitro* and *in vivo*. Thus, it is likely that the effects of AhR on T-cell activation and polarization involve its direct effects on T cells and its indirect effects on DCs and other APCs.

The AhR controls DC generation and activity through molecular mechanisms that are only beginning to be elucidated. Indeed, a better understanding of the role of the AhR in DC function is emerging as studies on the control of the transcriptional response of DCs to stimulation accumulate (Amit et al., 2009; Garber et al., 2012). These studies led to the identification of a set of transcription factors that plays a pivotal role in the control of a DC response, for example nuclear factor- κ B and activator protein-1. Although the AhR itself was not found to be among those transcription factors, it has been shown to interact with them and regulate their activity and degradation (Gillesby et al., 1997; Tian et al., 1999; Kim et al., 2000). Thus, it is possible that the AhR controls the activity of DCs by means of its ability to establish protein-protein interactions that result in the functional modulation of its interaction partner.

Regardless of the molecular mechanisms involved, the AhR has a significant effect on antigen presentation by DCs. After antigen uptake in the presence of DC-maturing stimuli, DCs upregulate the expression of the class II major histocompatibility complex, costimulatory molecules and cytokines that promote the polarization of T cells into specific populations (Banchereau et al., 2000; Guermonprez et al., 2002). AhR activation decreases the expression of class II major histocompatibility complex and costimulatory molecules and also the production of Th1 and Th17 polarizing cytokines by DCs (Mezrich et al., 2010; Nguyen et al., 2010; Quintana et al., 2010b). Indeed, AhR activation boosts the ability of DCs to promote the differentiation of regulatory T cells.

The ability of AhR signaling to boost the differentiation of FoxP3⁺ Tregs through effects on DC was first

described by Hauben et al. (2008). These initial findings were validated by additional investigators and involve at least two mechanisms. First, AhR activation was found to upregulate the expression of the indoleamine 2,3-dioxygenase (IDO) in DCs (Mezrich et al., 2010; Nguyen et al., 2010). This upregulation results in the production of kynurenine and kynurenine metabolites, immunosuppressive tryptophan metabolites that play an important role in the regulation of autoimmunity (Mellor and Munn, 2004; Platten et al., 2005; Puccetti and Grohmann, 2007) and tumor immunity and that have the potential to activate the AhR (Opitz et al., 2011). Indeed, kynurenine-derived metabolites boost the differentiation of FoxP3⁺ Tregs, and these effects are also dependent on AhR signaling in T cells. These findings suggest a positive feedback loop in which AhR ligands induce production of IDO, which in turn generates more kynurenine-derived endogenous AhR ligands (see Section IV).

In addition, AhR activation in DCs upregulates the enzymatic machinery that controls production of retinoic acid (RA) (Quintana et al., 2010b). RA promotes the differentiation of FoxP3⁺ Tregs, particularly in mucosal sites (Hall et al., 2011). Indeed, the differentiation of FoxP3⁺ Tregs induced by DCs in which AhR signaling has been initiated can be significantly suppressed by inhibition of RA signaling (Quintana et al., 2010b). Together with the reported role of the AhR in the regulation of gut immunity and the abundance of AhR ligands provided by the diet and the commensal flora, it is possible that the control of RA production in DCs by the AhR plays an important role in the control of mucosal immunity.

IV. Endogenous Aryl Hydrocarbon Receptor Ligands and Amplification of Aryl Hydrocarbon Receptor Activation with Positive Feedback Loops

Although the phenotypic consequences of AhR knock-out in mice initially appeared relatively subtle, subsequent studies demonstrated a clear role for the AhR in several normal physiologic processes, including development of the vasculature, construction of the central nervous system, differentiation of blood cell subsets, and the function of hepatocytes, adipocytes, and epithelial cells. As noted in the previous section, the AhR controls many processes in the development of Treg subsets, including activation of c-maf (through direct AhR-c-maf binding) and production of IL-10, Aiolos, and IL-21. In Th17 cells, the AhR promotes production of IL-21 and IL-22 and induces Aiolos activity. In DC and macrophages, it can influence expression of immunostimulatory as well as immunosuppressive cytokines and influences production of immunosuppressive tryptophan metabolites. The significance of all of these results with regard to AhR control of autoimmunity, tumor

immunity (a form of autoimmunity), and inflammation begs the question of what endogenous ligand(s) regulate AhR activation in situ. Indeed, they suggest that endogenous AhR ligand(s) are produced at key points in immune cell development and differentiation. That said, the identification of the exact nature of the endogenous AhR ligand(s) has remained elusive, likely confounded by the relative promiscuity of the AhR. Here, we present examples of several classes of AhR ligands, all of which qualify as candidate endogenous ligands involved in nominal AhR activation. Indeed, because this list of possible candidates is long, we propose the hypothesis that there are many endogenous AhR ligands, most or all of which contribute to AhR-dependent physiologic processes in a tissue- and context-specific manner. Because AhR activation often results in upregulation of enzymes involved in generating putative endogenous ligands, we postulate that AhR activation invokes a positive feedback loop that sustains or increases AhR activity in the local microenvironment (Fig. 2). Furthermore, we postulate that, as for xenobiotic AhR ligands, different endogenous AhR ligands induce different outcomes in the same or different tissues. This corollary becomes important when considering AhR modulators as therapeutics because AhR-directed therapeutics likely differ from one another and from environmental ligands in the outcomes they induce.

A. Indirect Evidence of Endogenous Aryl Hydrocarbon Receptor Ligands

One of the earliest results supporting the presence of endogenous AhR ligands came from studies demonstrating that CYP1A1-deficient cells exhibit a higher level of baseline AhR activity than wild-type cells (Chang and Puga, 1998). Ectopic expression of CYP1A1 decreases AhR activity to wild-type levels, suggesting that endogenous AhR ligand(s) are CYP1A1 substrates and that AhR-mediated CYP1A1 induction is, in effect, a negative feedback loop. Similarly, ectopic CYP1A2 and CYP1B1 expression decreases baseline AhR-dependent reporter activity (Chiaro et al., 2007). Additional studies using a CYP1A1-specific inhibitor, 1-(1-propynyl)pyrene, demonstrated that CYP1A1 downregulation increases AhR transcriptional activity in hepatoma cells (Levine-Fridman et al., 2004). We observed the same phenomenon after CYP1B1 knockdown in breast cancer cells (data not shown). The ability of CYP1A1 to degrade an endogenous AhR ligand was directly supported by the demonstration that CYP1A1 inhibition via ultraviolet B irradiation increases metabolism of 6-formylindolo[3,2-b]carbazole (FICZ), a postulated endogenous AhR ligand (Luecke et al., 2010; Wincent et al., 2012) (see section IV.D). Most recently, and with specific regard to the function of IL-22-secreting Th cells, it was shown that activation of the Notch signaling pathway induces production of an as yet unidentified AhR ligand capable of inducing T-cell-derived IL-22 (Alam et al.,

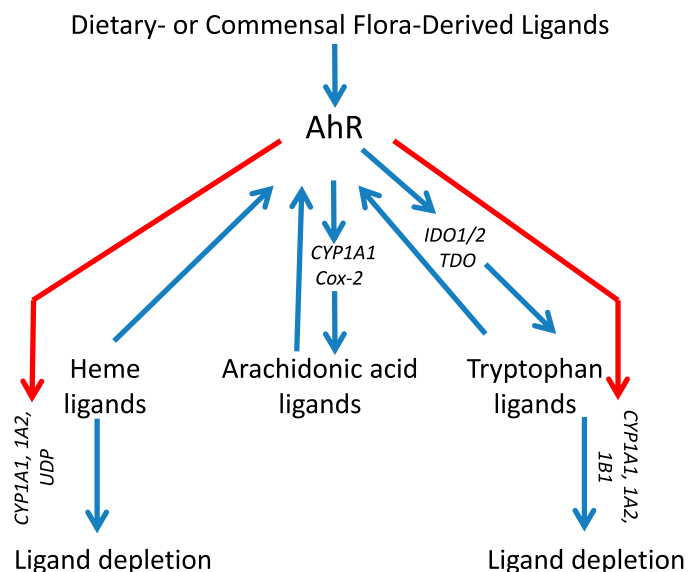


Fig. 2. Endogenous AhR ligands and amplification of AhR activation with positive feedback loops. Exogenous ligands derived from the diet or commensal flora may initiate AhR activation. Within any given cell, heme-, arachidonic acid-, or tryptophan-derived metabolites bind to and activate the AhR. Activated AhR transcriptionally upregulates expression of genes encoding enzymes involved in arachidonic acid (*CYP1A1*, *Cox-2*) and tryptophan (*CYP1A1*, *CYP1A2*, *CYP1B1*) metabolism, producing AhR ligands in a localized positive feedback loop. AhR upregulation of genes encoding enzymes that degrade heme-derived [*CYP1A1*, *CYP1A2*, *UDP-glucuronosyl-transferase (UDT)*] and tryptophan-derived (*CYP1A1*, *CYP1A2*, *CYP1B1*) AhR ligands represents a negative feedback loop.

2010). It was suggested that the source of this AhR ligand was T cells, although an increased level of AhR-inducing activity was observed when Notch signaling was induced in cocultures of T cells and APC compared with T cells alone. The identity of the putative AhR ligand(s) in these T-cell studies was not determined, although it was postulated to be a heat-labile molecule unrelated to tryptophan metabolites, previously suggested to act as endogenous AhR ligands (see section IV.D).

Perhaps the most compelling indirect evidence of endogenous, or at least “natural” AhR ligands, comes from studies with AhR^{-/-} mice in which several developmental defects are seen including a decrease in oocyte atresia (Robles et al., 2000; Matikainen et al., 2001, 2002), ductus venosus (Lahvis et al., 2005), immature vasculature development (Lahvis et al., 2005), cardiac hypertrophy (Fernandez-Salguero et al., 1997), oculomotor defects (Chevallier et al., 2013), disproportionate red blood cell and megakaryocyte production (Lindsey and Papoutsakis, 2011), altered hematopoietic stem cell differentiation (Singh et al., 2011a), and malformation of the gut epithelial barrier (Kiss et al., 2011; Li et al., 2011). Most relevant to T-cell development and function is the failure of AhR^{-/-} mice to generate gut-associated lymphoid leukocytes, including IL-22-secreting innate immune cells, and intraepithelial lymphocytes in both the gut and skin (Kiss et al., 2011; Li et al., 2011). Given the diversity of the

effects of AhR deficiency, it seems reasonable to propose either that a single AhR ligand is pervasive throughout the developing or mature animal or, more likely, that multiple AhR ligands, potentially derived from disparate sources, act on specific tissue in a highly regulated fashion and resulting in different outcomes.

B. Heme-Derived Molecules

The finding that rats defective in UDP-glucuronosyltransferase, a key bilirubin-degrading enzyme, have elevated CYP1A1 levels (Kapitulnik and Gonzalez, 1993) hinted at the ability of this, or related heme-derived metabolites, to activate the AhR. Indeed, nanomolar concentrations of bilirubin induce CYP1A1 in mouse hepatoma cells in an AhR-dependent manner (Sinal and Bend, 1997). A related heme-degradation product, biliverdin, also activates the AhR (Phelan et al., 1998). Because the bulk of bilirubin is conjugated to albumin and thereby unavailable as an AhR ligand, its effects may be local rather than systemic. Therefore, if either biliverdin or bilirubin in fact regulates the AhR in situ, it would be expected that they do so in heme-rich organs such as liver and spleen. Interestingly, CYP1A1 and CYP1A2, along with AhR-regulated UDP-glucuronosyltransferase, contribute to bilirubin catabolism (Kapitulnik and Gonzalez, 1993; Munzel et al., 2003; Erichsen et al., 2008; Buckley and Klaassen, 2009; Bock and Kohle, 2010), suggesting a negative feedback loop consistent with that postulated by Chang and Puga (1998) and Levine-Fridman et al., 2004 (Fig. 2).

C. Arachidonic Acid Metabolites

For many years, investigators have reported an apparent correlation between outcomes seen in AhR^{-/-} mice or after xenobiotic activation of the AhR and functions controlled by arachidonic acid metabolites, particularly inflammatory responses (Nebert and Karp, 2008). However, the exact mechanisms responsible for this association have been difficult to define, although some studies suggest that AhR activation by eicosanoids is responsible. Eicosanoids, of which over 120 have been identified, are a set of biologically activate oxygenated derivatives of arachidonic acid produced, in part, as a consequence of CYP1A1 and COX-2 enzyme activity. It is noteworthy that both the *CYP1A1* and *COX-2* genes are well-established AhR gene targets (Degner et al., 2007, 2009; Vogel et al., 2007), thereby linking AhR activation to prostaglandin, prostacyclin, and thromboxane synthesis. Indeed, TCDD increases expression of four dihydroxyeicosatrienoic acids and four hydroxyeicosatrienoic acids (HETEs) in an AhR-dependent manner in vivo (Bui et al., 2012). Analogous to the heme-derived AhR ligands, arachidonic acid derivatives may participate in a positive feedback loop (Fig. 2) because several prostaglandins (PGF3 α , PGG2, PGH1, PGB3, PGD3, and PGH2), leukotrienes

(6-*trans*-LTB 4, 6- *trans*-12-epi-LTB), dihydroxyeicosatrienoic acids [4,5(*S*),6(*S*)-DiHETE, 5(*S*),6(*R*)-DiHETE], and at least one hydroxyeicosatrienoic acid ([12(*R*)-HETE]) and one lipoxin (lipoxin A4) are AhR inducers (Schaldach et al., 1999; Seidel et al., 2001; Chiaro et al., 2008a,b). Of these compounds, PGG2 and [12(*R*)-HETE] are the most potent and efficacious AhR activators and are capable of inducing AhR activity at nanomolar concentrations and, in some cases, inducing a 3- to 4-fold greater level of AhR-dependent reporter activity than TCDD (Seidel et al., 2001; Chiaro et al., 2008b). Nevertheless, even the weaker arachidonic acid metabolites may play a physiologic role in their respective local environments. Furthermore, the disparate molecular structure of these compounds, particularly lipoxin A4, which does not contain a ring structure, underscores the relative permissiveness of the AhR ligand-binding domain.

D. Tryptophan Metabolites

Perhaps the leading candidates for endogenous AhR ligands are tryptophan-derived metabolites. There are at least five pathways of tryptophan metabolism that have been implicated in the regulation of AhR activation:

1. The primary route of tryptophan metabolism in mammals is the kynurenine pathway. The most proximal metabolite, kynurenine, is produced through the activity of IDO1/2 or tryptophan-2,3-dioxygenase (Allegrì et al., 2003). Kynurenine is produced by glioblastomas and regulates their survival and motility through AhR signaling (Opitz et al., 2011). More germane to autoimmunity, kynurenine induces regulatory T cells (Mezrich et al., 2010), although its source in vivo is unknown. The downstream metabolites kynurenic acid and xanthurenic acid also act as AhR ligands (Heath-Pagliuso et al., 1998; DiNatale et al., 2010). Kynurenic acid induces production of inflammatory cytokines (DiNatale et al., 2010). Importantly, IDO1 and IDO2 can be upregulated upon AhR activation (Vogel et al., 2008; Bankoti et al., 2010), again suggesting that AhR activation may result in a positive feedback loop that prolongs and/or amplifies AhR signaling in the local environment (Fig. 2).
2. The IDO/tryptophan-2,3-dioxygenase-independent tryptamine pathway, catalyzed by tryptophan hydroxylase and dopamine decarboxylase, also has been implicated in AhR activation. The most proximal metabolite in that pathway, tryptamine, is a potent AhR activator and may be both a direct AhR ligand (Heath-Pagliuso et al., 1998) and a precursor of a downstream AhR ligand(s) (Vikstrom Bergander et al., 2012). Indeed, at least one metabolite downstream of tryptamine,

- indole acetic acid, is an AhR ligand (Heath-Pagliuso et al., 1998). Of note, tryptamine is a competitive CYP1A1 substrate, a result consistent with the prediction that endogenous AhR ligand(s) is degraded by CYP1 enzymes (Chiaro et al., 2007).
3. The serotonin pathway may be directly or indirectly connected to AhR activation. 5-Hydroxytryptophan, a proximal serotonin metabolite, is an AhR agonist of modest potency (Bittinger et al., 2003). In our hands, serotonin itself is a weak AhR activator in an AhR-dependent reporter assay (data not shown). Perhaps more important to AhR regulation is the metabolic pathway distal to serotonin. In one branch of the serotonin pathway represented by tryptophan → 5-hydroxy-L-tryptophan → serotonin → *N*-acetylserotonin → melatonin → 6-hydroxymelatonin, melatonin conversion is catalyzed by CYP1A1, CYP1A2, and CYP1B1. Therefore, upregulation of CYP1 enzymes could result in a metabolic sink that depletes tryptophan stores and reduces production of endogenous AhR ligands in the kynurenine and/or tryptamine pathways (Fig. 2). Consequently, an increase in AhR activity after exposure to a given compound (Vikstrom Bergander et al., 2012) could be mistaken for evidence that the compound is an AhR ligand when in reality the increase in AhR activity could be because of blocking of the serotonin pathway metabolic sink and the subsequent increase in concentration of AhR ligands from the kynurenine, tryptamine, or tryptophan photometabolite (see below) pathways.
 4. It was first suggested in 1987 that tryptophan photometabolites represent endogenous AhR ligands (Rannug et al., 1987). Since then, many studies have demonstrated that 6-formylindolo [3,2-*b*]carbazole (FICZ) binds to the AhR with high affinity and initiates AhR signaling at nanomolar concentrations (Rannug et al., 1987; Wei et al., 1999, 2000; Oberg et al., 2005; Goryo et al., 2007; Mukai and Tischkau, 2007; Wincent et al., 2009, 2012). FICZ can contribute to (Kostyuk et al., 2012) or block (Jeong et al., 2012) inflammatory responses in a tissue- and context-specific fashion. FICZ also influences responses to ultraviolet light, genomic stability, and circadian rhythms, all postulated to be AhR mediated (Wincent et al., 2012). At least one additional tryptophan photometabolite, 1-(1*H*-indol-3-yl)-9*H*-pyrido[3,4-*b*]indole, has been identified and shown to exhibit a similar potency and efficacy as FICZ (Diani-Moore et al., 2011). Although initially thought to exert only local AhR-mediated effects in the skin, the influence of these photometabolites may be systemic as FICZ has been found in human urine (Wincent et al., 2009). Finally, FICZ is readily metabolized by CYP1A1, CYP1A2, and CYP1B1 (Wincent et al., 2012), satisfying a proposed criterion for endogenous AhR ligands (Diani-Moore et al., 2011).
 5. Plant-derived phytochemicals were among the earliest of postulated “natural” AhR ligands. Plant-derived indirubin and indigo are AhR ligands of relatively high potency (Adachi et al., 2001). However, the extremely low concentration in which they are found in human urine (~0.2 nM) and the failure to detect either compound in mammalian tissue suggests that they are not meaningful AhR inducers under normal physiologic conditions. Indole-3-carbinol and its condensation derivatives 3,3'-diindolmethane, indolo [3,2-*b*]carbazole, and 2-(indol-3-ylmethyl)-3,3'-diindolymethane, are derived from cruciferous vegetables and exhibit strong affinity for the AhR (Bjeldanes et al., 1991; Chen et al., 1996, 1998; Safe et al., 1999; Safe and McDougal, 2002; Ociepa-Zawal et al., 2007; Degner et al., 2009). These or other phytochemicals are very likely to be important in the context of T-cell development, autoimmunity, and inflammation inasmuch as dietary AhR ligands are critical for the development of gut-associated intraepithelial lymphocytes and maintenance of gut epithelial barrier integrity (Kiss et al., 2011; Li et al., 2011). Furthermore, the involvement of yet more tryptophan/indole derivatives in AhR activation suggests another intriguing possibility that indole, produced from tryptophan by a variety of commensal gut microflora, serves as the precursor for the generation of one or more “endogenous” AhR ligands. If confirmed, this would directly tie the microbiome to production of AhR ligands capable of influencing T-cell development and function at multiple levels.

E. Aryl Hydrocarbon Receptor Feedback Loops

From the results described above, it is apparent that control of the production and degradation of endogenous AhR ligands is complex (Fig. 2). The likelihood of positive feedback loops could significantly amplify the signal induced even by weak AhR ligands. Their existence also suggests the possibility that, once homeostasis is perturbed by exposure to low levels of environmental AhR ligands or AhR-targeted therapeutics, a self-perpetuating loop of AhR signaling may persist, even if the initiating ligand is metabolized and cleared. Although this may be undesirable in the context of environmental chemical exposures, it may be useful for therapeutic strategies aimed at activating the AhR. The fact that the AhR induces enzymes that degrade some endogenous AhR ligands also adds a level of complexity and unpredictability. Compounds that bind to and activate the AhR also tend to be substrates

of AhR-regulated CYP1 enzymes, e.g., tryptamine (Heath-Pagliuso et al., 1998), FICZ (Wei et al., 2000), and indole-3-carbinol derivatives (Bittinger et al., 2003). Indeed, it has been suggested that some compounds that induce AhR signaling do so not because they are AhR ligands but because they reduce catabolism of bona fide endogenous ligands (Wincent et al., 2012). Consequently, the magnitude and persistence of AhR signaling is a function of the affinity of a compound for the AhR, its activity as a CYP1 substrate (or inhibitor), the AhR-independent factors that control CYP1 levels in any given cell type or tissue, and the propensity for the AhR in any given tissue to amplify expression of enzymes involved in endogenous ligand synthesis. Therefore, predictions of what any particular AhR ligand may do in a given context remain difficult to make. Therefore, empirical testing may be the only way to determine if any given AhR ligand, e.g., an AhR-targeted therapeutic, induces any AhR-mediated toxicity.

V. Aryl Hydrocarbon Receptor as a Therapeutic Target

Because of its effects on the regulatory T-cell compartment, the AhR is considered a potential therapeutic target for the treatment of autoimmune disorders. Indeed, the administration of certain AhR ligands, including TCDD, the quintessential environmental AhR ligand, leads to the expansion of functional Tregs that arrest the development of experimental autoimmune encephalomyelitis (EAE) (Quintana et al., 2008), experimental autoimmune uveoretinitis (Zhang et al., 2009), colitis (Benson and Shepherd, 2011; Singh et al., 2011b), and spontaneous autoimmune diabetes (Kerkvliet et al., 2009). Some limitations, however, will have to be overcome to translate these findings to the treatment of human autoimmune disorders.

One of these limitations is the broad tissue expression pattern of the AhR, potentially making it difficult to selectively target specific tissues after systemic administration of AhR modulators. Of particular concern for autoimmunity is the observation that development of both inflammatory Th17 cells, which promote organ-specific autoimmunity, and Treg cells, which oppose autoimmunity, are facilitated by AhR activation. Thus, systemically administered AhR ligands could, under different circumstances, exacerbate or ameliorate autoimmunity. To overcome this uncertainty, we have constructed nanoparticles (NPs) with the goal of delivering AhR ligands directly to APCs (Fig. 3). Nanotechnology offers new opportunities for therapeutic intervention in immune-mediated diseases. NP-based drug delivery systems increase the half-life of compounds, facilitating the delivery of one or several compounds to specific cell-types in vivo (Sanvicens and Marco, 2008). Based on the tolerogenic effects of AhR activation in

DCs, we engineered NPs to co-deliver the AhR ligand 2-(1*H*-indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE), which induces a tolerogenic phenotype in DCs in vitro, together with tissue-specific antigens (Yeste et al., 2012).

NPs protected ITE from degradation by liver enzymes, increasing its half-life in vivo (Yeste et al., 2012). After intravenous administration, ITE-loaded NPs activated AhR signaling in DCs in vivo. NP-triggered AhR activation in DCs induces a tolerogenic phenotype characterized by the reduced ability to generate Th1 and Th17 cells, concomitant with an increased ability to promote FoxP3⁺ Treg differentiation. Accordingly, NPs loaded with ITE and myelin antigens expand central nervous system-specific FoxP3⁺ Tregs that suppress EAE, both in preventive and therapeutic paradigms. Taken together, these data suggest that NP-based administration offers a new avenue for the selective activation of AhR in specific cell populations for therapeutic purposes.

A second issue that must be addressed when considering the use of AhR agonists for treatment of autoimmune disorders, or other diseases for that matter, is our lack of understanding of how different AhR ligands induce different outcomes. For example, in the same animal model of EAE, TCDD, or ITE treatment induces Treg cells and suppresses autoimmunity, whereas another ligand, FICZ, produces the opposite effect, i.e., it induces Th17 cells and exacerbates disease (Quintana et al., 2008). Notably, both ITE and FICZ may be physiologically relevant endogenous AhR ligands (Song et al., 2002; Wincent et al., 2009), suggesting the possibility that different ligands are generated in situ in a context-specific manner to induce different AhR-dependent outcomes.

In the field of toxicology, the ability of different AhR ligands to induce different biologic outcomes has been well established. Most frequently, these differences have been attributed to differences in ligand persistence, a function of ligand metabolism. For example, 7,12-dimethylbenz[*a*]anthracene is readily metabolized by AhR-induced monooxygenases (CYP1A1, CYP1A2, CYP1B1) and induces significant bone marrow toxicity in vivo and in vitro through apoptosis induction (Yamaguchi et al., 1997; Mann et al., 1999; Allan et al., 2003; Ryu et al., 2005; Teague et al., 2010). TCDD, which is not readily metabolized and, consequently, persists in vivo for 7–10 years, does not induce apoptosis in the bone marrow. Differences in biologic outcomes with different AhR ligands also have been attributed to different ligand affinities for the AhR. However, it is unlikely that affinity differences alone account for the opposite effects of, for example, TCDD and FICZ, because both of these compounds bind the AhR with comparable affinities ($K_d = 0.07$ – 1.7 nM) (Furuhashi et al., 1986; Wei et al., 2000; Nguyen and Bradfield, 2008).

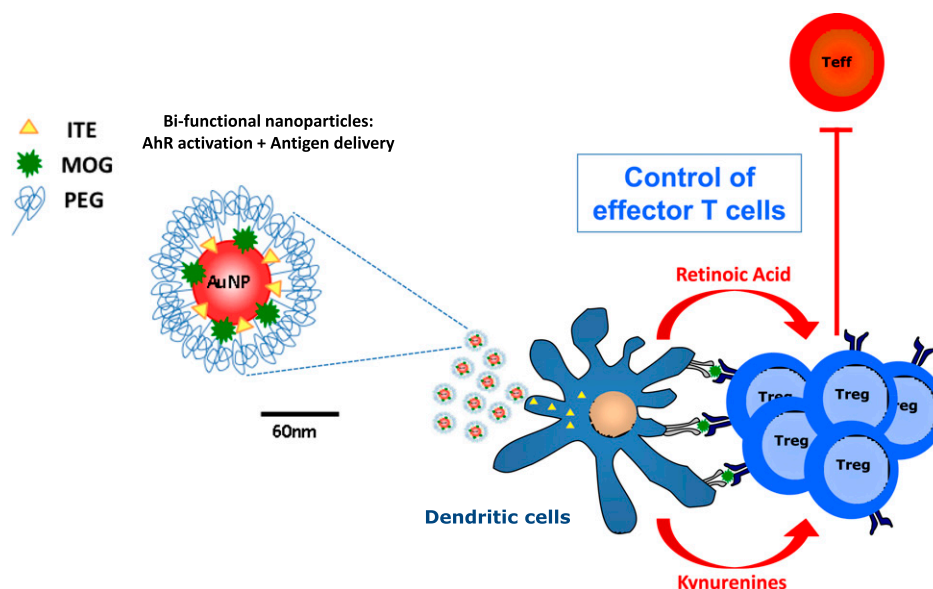


Fig. 3. Bi-functional nanoparticles for the induction of antigen-specific immune tolerance. Gold nanoparticles coated with polyethylene glycol (PEG) are engineered ligand ITE and the central nervous system antigen myelin oligodendrocyte protein (MOG) to dendritic cells in vivo. Dendritic cells that produce kynurenines (postulated tryptophan-derived endogenous AhR ligands) and retinoic acid induce and/or expand MOG-specific regulatory T cells (Treg) through retinoic acid receptor- and AhR-dependent signaling pathways. Treg cells actively suppress effector T-cell function (Teff), thereby inhibiting central nervous system autoimmunity.

This variability in biologic outcomes with different AhR ligands has another important implication, i.e., not all AhR ligands are toxic and/or carcinogenic. For example, although readily metabolized polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, and poorly metabolized TCDD are both toxic and carcinogenic, there is no evidence to suggest that any of the “natural” AhR ligands, including FICZ, ITE, eicosanoids, or kynurenine pathway metabolites, all of which induce CYP1 enzymes, are either toxic or carcinogenic. Therefore, an AhR-targeted therapeutic need not invoke the specter of unacceptable toxic side effects.

VI. Conclusions

AhR modulates the activity of the immune system in response to structurally heterogeneous ligands of diverse origin. These features of AhR are reminiscent of the effects on immunity of Toll-like receptor (TLR) signaling triggered by self and non-self agonists (Kawai and Akira, 2010). For example, both AhR and TLRs are evolutionary conserved systems that control innate and adaptive immunity in response to diverse environmental and endogenous cues. Indeed, AhR and TLR signaling intersect and cross-regulate each other. TLR activation upregulates AhR expression (Amit et al., 2009; Nguyen et al., 2010a), and AhR acts as a negative regulator of TLR signaling (Kimura et al., 2009; Mezrich et al., 2010; Nguyen et al., 2010; Quintana et al., 2010b). Thus, AhR, TLRs, and additional signaling pathways cross-talk to adjust the activity of the immune system in response to signals provided by the environment,

the diet, and the commensal flora. A corollary to this interpretation is that molecular targeting of the AhR, like molecular targeting of TLRs, provides an opportunity for therapeutic modulation of the immune response.

Several ligands operate in vivo, probably in a tissue-specific manner, to activate the AhR. The specific combination of the cytokine milieu, the specific tissue and ligand involved, determines whether the activation of AhR results in the promotion or the suppression of a specific immune response. Thus, controlled therapeutic targeting of the AhR requires the identification of ligands that promote the desired immune response without unwanted toxic or carcinogenic effects. Immunomodulatory endogenous ligands that lack toxicity and carcinogenicity have already been identified, suggesting that it is possible to design AhR-targeting compounds for the therapeutic modulation of the immune response. The half-life and therapeutic efficacy of these non-toxic AhR ligands might be increased with the use of nanomaterials for the targeted activation of AhR in specific cell types.

In conclusion, the AhR controls important immune processes in response to endogenous and environmental cues. Consequently, the AhR and its signaling pathway offer plausible molecular mechanisms through which environmental and endogenous ligands may control immunity and autoimmunity while providing us with new opportunities for targeted, therapeutic modulation of the immune response.

Acknowledgments

The authors want to thank the members of their laboratories for valuable discussions.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Quintana and Sherr.

References

- Adachi J, Mori Y, Matsui S, Takigami H, Fujino J, Kitagawa H, Miller CA 3rd, Kato T, Saeki K, and Matsuda T (2001) Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *J Biol Chem* **276**:31475–31478.
- Alam MS, Maekawa Y, Kitamura A, Tanigaki K, Yoshimoto T, Kishihara K, and Yasutomo K (2010) Notch signaling drives IL-22 secretion in CD4+ T cells by stimulating the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* **107**:5943–5948.
- Allan LL, Mann KK, Matulka RA, Ryu HY, Schlezinger JJ, and Sherr DH (2003) Bone marrow stromal-B cell interactions in polycyclic aromatic hydrocarbon-induced pro/pre-B cell apoptosis. *Toxicol Sci* **76**:357–365.
- Allegrì G, Bertazzo A, Biasiolo M, Costa CV, and Ragazzi E (2003) Kynurenine pathway enzymes in different species of animals. *Adv Exp Med Biol* **527**:455–463.
- Amit I, Garber M, Chevrier N, Leite AP, Donner Y, Eisenhaure T, Guttman M, Grenier JK, Li W, and Zuk O, et al. (2009) Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* **326**:257–263.
- Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, and Kuchroo VK (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol* **11**:854–861.
- Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, and Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol* **8**:1380–1389.
- Bach JF (2005) Infections and autoimmune diseases. *J Autoimmun* **25** (Suppl):74–80.
- Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, and Palucka K (2000) Immunobiology of dendritic cells. *Annu Rev Immunol* **18**:767–811.
- Bankoti J, Rase B, Simones T, and Shepherd DM (2010) Functional and phenotypic effects of AhR activation in inflammatory dendritic cells. *Toxicol Appl Pharmacol* **246**:18–28.
- Benson JM and Shepherd DM (2011) Aryl hydrocarbon receptor activation by TCDD reduces inflammation associated with Crohn's disease. *Toxicol Sci* **120**:68–78.
- Bettini ML, Pan F, Bettini M, Finkelstein D, Rehg JE, Floess S, Bell BD, Ziegler SF, Huehn J, and Pardoll DM, et al. (2012) Loss of epigenetic modification driven by the Foxp3 transcription factor leads to regulatory T cell insufficiency. *Immunity* **36**:717–730.
- Bittinger MA, Nguyen LP, and Bradford CA (2003) Aspartate aminotransferase generates progenitors of the aryl hydrocarbon receptor. *Mol Pharmacol* **64**:550–556.
- Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, and Bradford CA (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci USA* **88**:9543–9547.
- Bock KW and Köhle C (2010) Contributions of the Ah receptor to bilirubin homeostasis and its antioxidative and atheroprotective functions. *Biol Chem* **391**:645–653.
- Boronat S, Casado S, Navas JM, and Piña B (2007) Modulation of aryl hydrocarbon receptor transactivation by carbaryl, a nonconventional ligand. *FEBS J* **274**:3327–3339.
- Buckley DB and Klaassen CD (2009) Induction of mouse UDP-glucuronosyltransferase mRNA expression in liver and intestine by activators of aryl-hydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, peroxisome proliferator-activated receptor alpha, and nuclear factor erythroid 2-related factor 2. *Drug Metab Dispos* **37**:847–856.
- Bui P, Solaimani P, Wu X, and Hankinson O (2012) 2,3,7,8-Tetrachlorodibenzo-p-dioxin treatment alters eicosanoid levels in several organs of the mouse in an aryl hydrocarbon receptor-dependent fashion. *Toxicol Appl Pharmacol* **259**:143–151.
- Chang CY and Puga A (1998) Constitutive activation of the aromatic hydrocarbon receptor. *Mol Cell Biol* **18**:525–535.
- Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, Jack RS, Wunderlich FT, Brünig JC, and Müller W, et al. (2011) Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* **34**:566–578.
- Chen I, McDougal A, Wang F, and Safe S (1998) Aryl hydrocarbon receptor-mediated antiestrogenic and antitumor activity of diindolylmethane. *Carcinogenesis* **19**:1631–1639.
- Chen I, Safe S, and Bjeldanes L (1996) Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells. *Biochem Pharmacol* **51**:1069–1076.
- Chen Y, Haines CJ, Gutcher I, Hochweller K, Blumenschein WM, McClanahan T, Hämmerling G, Li MO, Cua DJ, and McGeachy MJ (2011) Foxp3(+) regulatory T cells promote T helper 17 cell development in vivo through regulation of interleukin-2. *Immunity* **34**:409–421.
- Chevallier A, Mialot A, Petit JM, Fernandez-Salguero P, Barouki R, Coumoul X, and Beranek M (2013) Oculomotor deficits in aryl hydrocarbon receptor null mouse. *PLoS ONE* **8**:e53520.
- Chiaro CR, Morales JL, Prabhu KS, and Perdew GH (2008a) Leukotriene A4 metabolites are endogenous ligands for the Ah receptor. *Biochemistry* **47**:8445–8455.
- Chiaro CR, Patel RD, Marcus CB, and Perdew GH (2007) Evidence for an aryl hydrocarbon receptor-mediated cytochrome P450 autoregulatory pathway. *Mol Pharmacol* **72**:1369–1379.
- Chiaro CR, Patel RD, and Perdew GH (2008b) 12(R)-Hydroxy-5(Z),8(Z),10(E),14(Z)-eicosatetraenoic acid [12(R)-HETE], an arachidonic acid derivative, is an activator of the aryl hydrocarbon receptor. *Mol Pharmacol* **74**:1649–1656.
- Christen U and von Herrath MG (2005) Infections and autoimmunity—good or bad? *J Immunol* **174**:7481–7486.
- Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, Agarwal A, Huang W, Parkurst CN, and Muratet M, et al. (2012) A validated regulatory network for Th17 cell specification. *Cell* **151**:289–303.
- Comabella M, Montalban X, Münz C, and Lünemann JD (2010) Targeting dendritic cells to treat multiple sclerosis. *Nat Rev Neurol* **6**:499–507.
- Degner SC, Kemp MQ, Hockings JK, and Romagnolo DF (2007) Cyclooxygenase-2 promoter activation by the aromatic hydrocarbon receptor in breast cancer mcf-7 cells: repressive effects of conjugated linoleic acid. *Nutr Cancer* **59**:248–257.
- Degner SC, Papoutsis AJ, Selmin O, and Romagnolo DF (2009) Targeting of aryl hydrocarbon receptor-mediated activation of cyclooxygenase-2 expression by the indole-3-carbinol metabolite 3,3'-diindolylmethane in breast cancer cells. *J Nutr* **139**:26–32.
- Diani-Moore S, Ma Y, Labitzke E, Tao H, David Warren J, Anderson J, Chen Q, Gross SS, and Rifkind AB (2011) Discovery and biological characterization of 1-(1H-indol-3-yl)-9H-pyrido[3,4-b]indole as an aryl hydrocarbon receptor activator generated by photoactivation of tryptophan by sunlight. *Chem Biol Interact* **193**:119–128.
- DiNatale BC, Murray IA, Schroeder JC, Flaveny CA, Lahoti TS, Laurenzana EM, Omiecinski CJ, and Perdew GH (2010) Kynurenine acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicol Sci* **115**:89–97.
- Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L, Takahashi H, Sun H-W, Kanno Y, and Powrie F, et al. (2010) Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* **32**:605–615.
- Erichsen TJ, Ehmer U, Kalthoff S, Lankisch TO, Müller TM, Munzel PA, Manns MP, and Strassburg CP (2008) Genetic variability of aryl hydrocarbon receptor (AhR)-mediated regulation of the human UDP glucuronosyltransferase (UGT) 1A4 gene. *Toxicol Appl Pharmacol* **230**:252–260.
- Fernandez-Salguero PM, Ward JM, Sundberg JP, and Gonzalez FJ (1997) Lesions of aryl-hydrocarbon receptor-deficient mice. *Vet Pathol* **34**:605–614.
- Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, Saris CJ, Gran B, Ciric B, and Rostami A (2007) Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat Immunol* **8**:1372–1379.
- Fontenot JD, Gavin MA, and Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* **4**:330–336.
- Fu W, Ergun A, Lu T, Hill JA, Haxhinasto S, Fasset MS, Gazit R, Adoro S, Glimcher L, and Chan S, et al. (2012) A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. *Nat Immunol* **13**:972–980.
- Furuhashi N, Kurl RN, Wong J, and Vilee CA (1986) A cytosolic binding protein for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the uterus and deciduoma of rats. *Pharmacology* **33**:110–120.
- Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL, and Quintana FJ (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. *Nat Immunol* **11**:846–853.
- Garber M, Yosef N, Goren A, Raychowdhury R, Thielke A, Guttman M, Robinson J, Minie B, Chevrier N, and Itzhaki Z, et al. (2012) A high-throughput chromatin immunoprecipitation approach reveals principles of dynamic gene regulation in mammals. *Mol Cell* **47**:810–822.
- Gillesby BE, Stanostefano M, Porter W, Safe S, Wu ZF, and Zacharewski TR (1997) Identification of a motif within the 5' regulatory region of pS2 which is responsible for AP-1 binding and TCDD-mediated suppression. *Biochemistry* **36**:6080–6089.
- Goryo K, Suzuki A, Del Carpio CA, Siizaki K, Kuriyama E, Mikami Y, Kinoshita K, Yasumoto K, Rannag A, and Miyamoto A, et al. (2007) Identification of amino acid residues in the Ah receptor involved in ligand binding. *Biochem Biophys Res Commun* **354**:396–402.
- Gottschalk RA, Corse E, and Allison JP (2012) Expression of Helios in peripherally induced Foxp3+ regulatory T cells. *J Immunol* **188**:976–980.
- Gregersen PK and Olsson LM (2009) Recent advances in the genetics of autoimmune disease. *Annu Rev Immunol* **27**:363–391.
- Gregori S, Roncarolo MG, and Bacchetta R (2011) Methods for in vitro generation of human type 1 regulatory T cells. *Methods Mol Biol* **677**:31–46.
- Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, and Roncarolo MG (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**:737–742.
- Guermontprez P, Valladeau J, Zitvogel L, Théry C, and Amigorena S (2002) Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* **20**:621–667.
- Hall JA, Grainger JR, Spencer SP, and Belkaid Y (2011) The role of retinoic acid in tolerance and immunity. *Immunity* **35**:13–22.
- Halliday GM, Damian DL, Rana S, and Byrne SN (2012) The suppressive effects of ultraviolet radiation on immunity in the skin and internal organs: implications for autoimmunity. *J Dermatol Sci* **66**:176–182.
- Hankinson O (2005) Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. *Arch Biochem Biophys* **433**:379–386.
- Hauben E, Gregori S, Draghici E, Migliaiavacca B, Olivieri S, Woisetschläger M, and Roncarolo MG (2008) Activation of the aryl hydrocarbon receptor promotes allograft-specific tolerance through direct and dendritic cell-mediated effects on regulatory T cells. *Blood* **112**:1214–1222.
- Heath-Pagliuso S, Rogers WJ, Tullis K, Seidel SD, Cenijn PH, Brouwer A, and Denison MS (1998) Activation of the Ah receptor by tryptophan and tryptophan metabolites. *Biochemistry* **37**:11508–11515.
- Hershko A and Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* **67**:425–479.

- Hori S, Nomura T, and Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**:1057–1061.
- Huang G and Elferink CJ (2012) A novel nonconsensus xenobiotic response element capable of mediating aryl hydrocarbon receptor-dependent gene expression. *Mol Pharmacol* **81**:338–347.
- Jeong KT, Hwang SJ, Oh GS, and Park JH (2012) FICZ, a tryptophan photoproduct, suppresses pulmonary eosinophilia and Th2-type cytokine production in a mouse model of ovalbumin-induced allergic asthma. *Int Immunopharmacol* **13**:377–385.
- Josefowicz SZ, Lu LF, and Rudensky AY (2012) Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* **30**:531–564.
- Kamratt T, Göggel R, and Erb KJ (2005) Induction, exacerbation and inhibition of allergic and autoimmune diseases by infection. *Trends Immunol* **26**:260–267.
- Kapitulnik J and Gonzalez FJ (1993) Marked endogenous activation of the CYP1A1 and CYP1A2 genes in the congenitally jaundiced Gunn rat. *Mol Pharmacol* **43**:722–725.
- Kawai T and Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* **11**:373–384.
- Kerkvliet NI, Baecher-Steppan L, Smith BB, Youngberg JA, Henderson MC, and Buhler DR (1990) Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57Bl/6 mice congenic at the Ah locus. *Fundam Appl Toxicol* **14**:532–541.
- Kerkvliet NI, Shepherd DM, and Baecher-Steppan L (2002) T lymphocytes are direct, aryl hydrocarbon receptor (AhR)-dependent targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): AhR expression in both CD4+ and CD8+ T cells is necessary for full suppression of a cytotoxic T lymphocyte response by TCDD. *Toxicol Appl Pharmacol* **185**:146–152.
- Kerkvliet NI, Steppan LB, Vorachek W, Oda S, Farrer D, Wong CP, Pham D, and Mourich DV (2009) Activation of aryl hydrocarbon receptor by TCDD prevents diabetes in NOD mice and increases Foxp3+ T cells in pancreatic lymph nodes. *Immunotherapy* **1**:539–547.
- Kim DW, Gazourian L, Quadri SA, Romieu-Mourez R, Sherr DH, and Sonenshein GE (2000) The RelA NF- κ B subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* **19**:5498–5506.
- Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, and Kishimoto T (2009) Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med* **206**:2027–2035.
- Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, and Kishimoto T (2008) Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc Natl Acad Sci USA* **105**:9721–9726.
- Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, and Diefenbach A (2011) Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science* **334**:1561–1565.
- Kivity S, Agmon-Levin N, Blank M, and Shoenfeld Y (2009) Infections and autoimmunity—friends or foes? *Trends Immunol* **30**:409–414.
- Kostyuk V, Potapovich A, Stancato A, De Luca C, Lulli D, Pastore S, and Korkina L (2012) Photo-oxidation products of skin surface squalene mediate metabolic and inflammatory responses to solar UV in human keratinocytes. *PLoS ONE* **7**:e44472.
- Kranich J, Maslowski KM, and Mackay CR (2011) Commensal flora and the regulation of inflammatory and autoimmune responses. *Semin Immunol* **23**:139–145.
- Lahvis GP, Pyzalski RW, Glover E, Pitot HC, McElwee MK, and Bradfield CA (2005) The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. *Mol Pharmacol* **67**:714–720.
- Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, Peterson DA, Stappenbeck TS, and Hsieh CS (2011) Peripheral education of the immune system by colonic commensal microbiota. *Nature* **478**:250–254.
- Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, Blank RB, Meylan F, Siegel R, and Hennighausen L, et al. (2007) Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* **26**:371–381.
- Lee JS, Cella M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, Mantovani A, Kopan R, Bradfield CA, and Newberry RD, et al. (2012a) AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nat Immunol* **13**:144–151.
- Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, Wu C, Kleinewietfeld M, Kunder S, and Hafler DA, et al. (2012b) Induction and molecular signature of pathogenic Th17 cells. *Nat Immunol* **13**:991–999.
- Levine-Fridman A, Chen L, and Elferink CJ (2004) Cytochrome P4501A1 promotes G1 phase cell cycle progression by controlling aryl hydrocarbon receptor activity. *Mol Pharmacol* **65**:461–469.
- Lewis KL and Reizis B (2012) Dendritic cells: arbiters of immunity and immunological tolerance. *Cold Spring Harb Perspect Biol* **4**:a007401.
- Li Y, Innocentini S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, and Veldhoen M (2011) Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell* **147**:629–640.
- Liao W, Lin JX, Wang L, Li P, and Leonard WJ (2011) Modulation of cytokine receptors by IL-2 broadly regulates differentiation into helper T cell lineages. *Nat Immunol* **12**:551–559.
- Lindsey S and Papoutsakis ET (2011) The aryl hydrocarbon receptor (AHR) transcription factor regulates megakaryocytic polyploidization. *Br J Haematol* **152**:469–484.
- Luecke S, Wincent E, Backlund M, Rannug U, and Rannug A (2010) Cytochrome P450 1A1 gene regulation by UVB involves crosstalk between the aryl hydrocarbon receptor and nuclear factor kappaB. *Chem Biol Interact* **184**:466–473.
- Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, Vieira AT, Kranich J, and Mackay CR (2012) Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. *Immunol Rev* **245**:164–176.
- Mann KK, Matulka RA, Hahn ME, Trombino AF, Lawrence BP, Kerkvliet NI, and Sherr DH (1999) The role of polycyclic aromatic hydrocarbon metabolism in dimethylbenz[a]anthracene-induced pre-B lymphocyte apoptosis. *Toxicol Appl Pharmacol* **161**:10–22.
- Marshall NB, Vorachek WR, Steppan LB, Mourich DV, and Kerkvliet NI (2008) Functional characterization and gene expression analysis of CD4+ CD25+ regulatory T cells generated in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Immunol* **181**:2382–2391.
- Matikainen T, Perez GI, Jurisicova A, Pru JK, Schlezinger JJ, Ryu HY, Laine J, Sakai T, Korsmeyer SJ, and Casper RF, et al. (2001) Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. *Nat Genet* **28**:355–360.
- Matikainen TM, Moriyama T, Morita Y, Perez GI, Korsmeyer SJ, Sherr DH, and Tilly JL (2002) Ligand activation of the aromatic hydrocarbon receptor transcription factor drives Bax-dependent apoptosis in developing fetal ovarian germ cells. *Endocrinology* **143**:615–620.
- Matsumura F (2009) The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. *Biochem Pharmacol* **77**:608–626.
- McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, and Cua DJ (2007) TGF- β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* **8**:1390–1397.
- McIntosh BE, Hogenesch JB, and Bradfield CA (2010) Mammalian Per-Arnt-Sim proteins in environmental adaptation. *Annu Rev Physiol* **72**:625–645.
- Mellor AL and Munn DH (2004) IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* **4**:762–774.
- Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, and Bradfield CA (2010) An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* **185**:3190–3198.
- Mora JR, Iwata M, and von Andrian UH (2008) Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* **8**:685–698.
- Mukai M and Tischkau SA (2007) Effects of tryptophan photoproducts in the circadian timing system: searching for a physiological role for aryl hydrocarbon receptor. *Toxicol Sci* **95**:172–181.
- Münzel PA, Schmöhl S, Buckler F, Jaehrling J, Raschko FT, Köhle C, and Bock KW (2003) Contribution of the Ah receptor to the phenolic antioxidant-mediated expression of human and rat UDP-glucuronosyltransferase UGT1A6 in Caco-2 and rat hepatoma 5L cells. *Biochem Pharmacol* **66**:841–847.
- Murray IA, Morales JL, Flaveny CA, Dinatale BC, Chiaro CR, Gowdahalli K, Amin SG, and Perdue GH (2010) Evidence for ligand-mediated selective modulation of aryl hydrocarbon receptor activity. *Mol Pharmacol* **77**:247–254.
- Nebert DW and Karp CL (2008) Endogenous functions of the aryl hydrocarbon receptor (AHR): intersection of cytochrome P450 1 (CYP1)-metabolized eicosanoids and AHR biology. *J Biol Chem* **283**:36061–36065.
- Nguyen LP and Bradfield CA (2008) The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* **21**:102–116.
- Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, and Kishimoto T (2010) Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci USA* **107**:19961–19966.
- Oberg M, Bergander L, Håkansson H, Rannug U, and Rannug A (2005) Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. *Toxicol Sci* **85**:935–943.
- Ociepa-Zawal M, Rubiś B, Laciński M, and Trzeciak WH (2007) The effect of indole-3-carbinol on the expression of CYP1A1, CYP1B1 and AhR genes and proliferation of MCF-7 cells. *Acta Biochim Pol* **54**:113–117.
- Oesch-Bartlomowicz B, Huelster A, Wiss O, Antoniou-Lipfert P, Dietrich C, Arand M, Weiss C, Bockamp E, and Oesch F (2005) Aryl hydrocarbon receptor activation by cAMP vs. dioxin: divergent signaling pathways. *Proc Natl Acad Sci USA* **102**:9218–9223.
- Ohtake F, Baba A, Takada I, Okada M, Iwasaki K, Miki H, Takahashi S, Kouzmenko A, Nohara K, and Chiba T, et al. (2007) Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. *Nature* **446**:562–566.
- Opitz CA, Litzemberger UM, Sahn F, Ott M, Tritschler I, Trump S, Schumacher T, Jestaedt L, Schrenk D, and Weller M, et al. (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* **478**:197–203.
- Pan F, Yu H, Dang EV, Barbi J, Pan X, Grosso JF, Jinasena D, Sharma SM, McCadden EM, and Getnet D, et al. (2009) Eos mediates Foxp3-dependent gene silencing in CD4+ regulatory T cells. *Science* **325**:1142–1146.
- Pandiyani P, Conti HR, Zheng L, Peterson AC, Mather DR, Hernández-Santos N, Edgerton M, Gaffen SL, and Lenardo MJ (2011) CD4(+)/CD25(+)/Foxp3(+) regulatory T cells promote Th17 cells in vivo and enhance host resistance in mouse *Candida albicans* Th17 cell infection model. *Immunity* **34**:422–434.
- Phelan D, Winter GM, Rogers WJ, Lam JC, and Denison MS (1998) Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. *Arch Biochem Biophys* **357**:155–163.
- Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, Gupta R, Lee LY, Kidd BA, and Robinson WH, et al. (2005) Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. *Science* **310**:850–855.
- Puccetti P and Grohmann U (2007) IDO and regulatory T cells: a role for reverse signalling and non-canonical NF- κ B activation. *Nat Rev Immunol* **7**:817–823.
- Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, and Weiner HL (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* **453**:65–71.
- Quintana FJ, Iglesias AH, Farez MF, Caccamo M, Burns EJ, Kassam N, Oukka M, and Weiner HL (2010a) Adaptive autoimmunity and Foxp3-based immunoregulation in zebrafish. *PLoS ONE* **5**:e9478.
- Quintana FJ, Jin H, Burns EJ, Nadeau M, Yeste A, Kumar D, Rangachari M, Zhu C, Xiao S, and Seavitt J, et al. (2012) Aiols promotes Th17 differentiation by directly silencing Il2 expression. *Nat Immunol* **13**:770–777.

- Quintana FJ, Murugaiyan G, Farez MF, Mitsdoerffer M, Tukpah AM, Burns EJ, and Weiner HL (2010b) An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* **107**:20768–20773.
- Rannug A, Rannug U, Rosenkranz HS, Winqvist L, Westerholm R, Agurell E, and Grafström AK (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *J Biol Chem* **262**:15422–15427.
- Robles R, Morita Y, Mann KK, Perez GI, Yang S, Matikainen T, Sherr DH, and Tilly JL (2000) The aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse. *Endocrinology* **141**:450–453.
- Rook GA (2011) Hygiene and other early childhood influences on the subsequent function of the immune system. *Dig Dis* **29**:144–153.
- Ryu HY, Emberley JK, Schlezinger JJ, Allan LL, Na S, and Sherr DH (2005) Environmental chemical-induced bone marrow B cell apoptosis: death receptor-independent activation of a caspase-3 to caspase-8 pathway. *Mol Pharmacol* **68**:1087–1096.
- Safe S and McDougal A (2002) Mechanism of action and development of selective aryl hydrocarbon receptor modulators for treatment of hormone-dependent cancers (Review). *Review Int J Oncol* **20**:1123–1128.
- Safe S, Qin C, and McDougal A (1999) Development of selective aryl hydrocarbon receptor modulators for treatment of breast cancer. *Expert Opin Invest Drugs* **8**:1385–1396.
- Sakaguchi S, Miyara M, Costantino CM, and Hafler DA (2010) FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* **10**:490–500.
- Sanvicens N and Marco MP (2008) Multifunctional nanoparticles—properties and prospects for their use in human medicine. *Trends Biotechnol* **26**:425–433.
- Saraiva M and O'Garra A (2010) The regulation of IL-10 production by immune cells. *Nat Rev Immunol* **10**:170–181.
- Satpathy AT, Wu X, Albring JC, and Murphy KM (2012) Re(de)fining the dendritic cell lineage. *Nat Immunol* **13**:1145–1154.
- Schalldach CM, Riby J, and Bjeldanes LF (1999) Lipoxin A4: a new class of ligand for the Ah receptor. *Biochemistry* **38**:7594–7600.
- Seidel SD, Winters GM, Rogers WJ, Ziccardi MH, Li V, Keser B, and Denison MS (2001) Activation of the Ah receptor signaling pathway by prostaglandins. *J Biochem Mol Toxicol* **15**:187–196.
- Sinal CJ and Bend JR (1997) Aryl hydrocarbon receptor-dependent induction of cyp1a1 by bilirubin in mouse hepatoma hepa 1c1c7 cells. *Mol Pharmacol* **52**:590–599.
- Singh KP, Garrett RW, Casado FL, and Gasiewicz TA (2011a) Aryl hydrocarbon receptor-null allele mice have hematopoietic stem/progenitor cells with abnormal characteristics and functions. *Stem Cells Dev* **20**:769–784.
- Singh NP, Singh UP, Singh B, Price RL, Nagarkatti M, and Nagarkatti PS (2011b) Activation of aryl hydrocarbon receptor (AhR) leads to reciprocal epigenetic regulation of FoxP3 and IL-17 expression and amelioration of experimental colitis. *PLoS ONE* **6**:e23522.
- Song J, Clagett-Dame M, Peterson RE, Hahn ME, Westler WM, Sicinski RR, and DeLuca HF (2002) A ligand for the aryl hydrocarbon receptor isolated from lung. *Proc Natl Acad Sci USA* **99**:14694–14699.
- Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, Turka LA, Ernst M, Saris CJ, O'Shea JJ, and Hunter CA (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat Immunol* **8**:1363–1371.
- Teague JE, Ryu HY, Kirber M, Sherr DH, and Schlezinger JJ (2010) Proximal events in 7,12-dimethylbenz[a]anthracene-induced, stromal cell-dependent bone marrow B cell apoptosis: stromal cell-B cell communication and apoptosis signaling. *J Immunol* **185**:3369–3378.
- Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, and Shevach EM (2010) Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J Immunol* **184**:3433–3441.
- Tian Y, Ke S, Denison MS, Rabson AB, and Gallo MA (1999) Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. *J Biol Chem* **274**:510–515.
- Veldhoen M and Brucklacher-Waldert V (2012) Dietary influences on intestinal immunity. *Nat Rev Immunol* **12**:696–708.
- Veldhoen M, Hirota K, Christensen J, O'Garra A, and Stockinger B (2009) Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J Exp Med* **206**:43–49.
- Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renaud JC, and Stockinger B (2008) The aryl hydrocarbon receptor links Th17-cell-mediated autoimmunity to environmental toxins. *Nature* **453**:106–109.
- Vikström Bergander L, Cai W, Klocke B, Seifert M, and Pongratz I (2012) Tryptamine serves as a proligand of the AhR transcriptional pathway whose activation is dependent of monoamine oxidases. *Mol Endocrinol* **26**:1542–1551.
- Vogel CF, Goth SR, Dong B, Pessah IN, and Matsumura F (2008) Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochem Biophys Res Commun* **375**:331–335.
- Vogel CF, Li W, Sciallo E, Newman J, Hammock B, Reader JR, Tuscano J, and Matsumura F (2007) Pathogenesis of aryl hydrocarbon receptor-mediated development of lymphoma is associated with increased cyclooxygenase-2 expression. *Am J Pathol* **171**:1538–1548.
- Wan YY and Flavell RA (2007) Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* **445**:766–770.
- Wei YD, Bergander L, Rannug U, and Rannug A (2000) Regulation of CYP1A1 transcription via the metabolism of the tryptophan-derived 6-formylindolo[3,2-b]carbazole. *Arch Biochem Biophys* **383**:99–107.
- Wei YD, Rannug U, and Rannug A (1999) UV-induced CYP1A1 gene expression in human cells is mediated by tryptophan. *Chem Biol Interact* **118**:127–140.
- Wincent E, Amini N, Luecke S, Glatt H, Bergman J, Crescenzi C, Rannug A, and Rannug U (2009) The suggested physiologic aryl hydrocarbon receptor activator and cytochrome P4501 substrate 6-formylindolo[3,2-b]carbazole is present in humans. *J Biol Chem* **284**:2690–2696.
- Wincent E, Bengtsson J, Mohammadi Bardbori A, Alsberg T, Luecke S, Rannug U, and Rannug A (2012) Inhibition of cytochrome P4501-dependent clearance of the endogenous agonist FICZ as a mechanism for activation of the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* **109**:4479–4484.
- Wu HY, Quintana FJ, da Cunha AP, Dake BT, Koeglperger T, Starosom SC, and Weiner HL (2011) In vivo induction of Tr1 cells via mucosal dendritic cells and AHR signaling. *PLoS ONE* **6**:e23618.
- Xu J, Yang Y, Qiu G, Lal G, Wu Z, Levy DE, Ochando JC, Bromberg JS, and Ding Y (2009) c-Maf regulates IL-10 expression during Th17 polarization. *J Immunol* **182**:6226–6236.
- Yamaguchi K, Near RI, Matulka RA, Shneider A, Toselli P, Trombino AF, and Sherr DH (1997) Activation of the aryl hydrocarbon receptor/transcription factor and bone marrow stromal cell-dependent preB cell apoptosis. *J Immunol* **158**:2165–2173.
- Yang XP, Ghoreschi K, Steward-Tharp SM, Rodriguez-Canales J, Zhu J, Grainger JR, Hirahara K, Sun HW, Wei L, and Vahedi G, et al. (2011) Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. *Nat Immunol* **12**:247–254.
- Yazdanbakhsh M, Kremsner PG, and van Ree R (2002) Allergy, parasites, and the hygiene hypothesis. *Science* **296**:490–494.
- Yeste A, Nadeau M, Burns EJ, Weiner HL, and Quintana FJ (2012) Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* **109**:11270–11275.
- Zhang L, Ma J, Takeuchi M, Usui Y, Hattori T, Okunuki Y, Yamakawa N, Kezuka T, Kuroda M, and Goto H (2010) Suppression of experimental autoimmune uveoretinitis by inducing differentiation of regulatory T cells via activation of aryl hydrocarbon receptor. *Invest Ophthalmol Vis Sci* **51**:2109–2117.
- Zhang S, Rowlands C, and Safe S (2008) Ligand-dependent interactions of the Ah receptor with coactivators in a mammalian two-hybrid assay. *Toxicol Appl Pharmacol* **227**:196–206.