The Aryl Hydrocarbon Receptor in Barrier Organ Physiology, Immunology, and Toxicology

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Abstract—The aryl hydrocarbon receptor (AhR) is an evolutionarily old transcription factor belonging to the Per-ARNT-Sim–basic helix-loop-helix protein family. AhR translocates into the nucleus upon binding of various small molecules into the pocket of its single-ligand binding domain. AhR binding to both xenobiotic and endogenous ligands results in highly cell-specific transcriptome changes and in changes in cellular functions. We discuss here the role of AhR for immune cells of the barrier organs: skin, gut, and lung. Both adaptive and innate immune cells require AhR signaling at critical checkpoints. We also discuss the current two prevailing views—namely, 1) AhR as a promiscuous sensor for small chemicals and 2) a role for AhR as a balancing factor for cell differentiation and function, which is controlled by levels of endogenous high-affinity ligands. AhR signaling is considered a promising drug and preventive target, particularly for cancer, inflammatory, and autoimmune diseases. Therefore, understanding its biology is of great importance.

I. Introduction

The environment offers both vital benefits, such as food and sunlight, and deadly risks, such as infectious pathogens and toxins. The role of epithelial barrier organs such as skin, gut, lung, and other mucosal tissues that interact with the environment is critical for survival. Furthermore, these barriers are involved in uptake and absorption of nutrients as well as protection of body integrity against chemical, biologic, and physical stress, and more. The aryl hydrocarbon receptor (AhR) is a latent cytoplasmic transcription factor that can be activated by certain low molecular weight chemicals. The AhR is highly expressed in many barrier organs and in the liver, and its expression pattern conceivably suggests a role as a sensor of chemicals. Many AhR-activating factors, which cause transcriptional activation, have been identified (Denison and Nagy, 2003). They range from environmental pollutants, such as polyhalogenated aromatic hydrocarbons (PHAHs), to endogenous amino acid derivatives, dietary chemicals such as indoles or glucosinolates, and finally natural substances found in yeasts and bacteria or even marine sponges. As demonstrated by in vitro studies and gene expression profiles, the transcriptional changes by the activated AhR are ligand specific and are highly cell specific. For a given cell type, they may even depend on the tissue milieu, such as an ongoing immune response (Sun et al., 2004; Frericks et al., 2007; Beischlag et al., 2008; Tappenden et al., 2013). There appears to be only one ligand binding pocket in the AhR (in the Per-ARNT-Sim-B domain), and the binding affinity of the best characterized high-affinity ligand, TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), depends on only a few amino acids in the binding pocket of the AhR, as demonstrated by modeling and mutation studies (Whelan et al., 2010; DeGroot et al., 2012; Wu et al., 2013). The AhR can also be activated by numerous stress factors and substances that may not fit into the binding pocket, such as hyperoxia, oxidized low-density lipoproteins, hydrogen peroxide, ozone, or metals (references in Wincent et al., 2012). Much of this is not understood because the AhR has not yet been crystallized, although it was cloned more than 2 decades ago (Burbach et al., 1992).

Research on the AhR has undergone a major paradigm shift in recent years. It has long focused on induction of genes coding for metabolizing enzymes, the so-called AhR-battery genes (Nebert et al., 1991; Köhle and Bock, 2007). In particular, cytochrome P450 was of interest because of the toxicity associated with its activity in adverse drug–drug interactions and generation of carcinogenic metabolites from polycyclic aromatic hydrocarbons (PAHs). Especially in the field of toxicology and pharmacology, the AhR is viewed as a protein that has developed during evolution to mediate metabolism of environmental small molecules. This view of the AhR as a promiscuous cytosolic sensor of small molecules that is primarily involved in biotransformation and detoxification is rapidly changing. As regarded today, the AhR plays an important role in cell development, differentiation, and function. Recent evidence from studies with full and conditional AhR-deficient animal models implies important endogenous roles for the AhR. These include control in perinatal growth, fertility, hepatic and vascular development, peripheral and intestinal immunity and hematopoiesis, as well as in stem cell expansion and cancer. The specific ligands that are drivers of activation in a given situation are not fully clear.

It is recognized that xenobiotic small chemicals can aberrantly activate AhR because of their structural likeness to physiologic ligands. By this process, chemicals may increase the metabolic turnover of physiologic ligands and thereby decrease the half-life of the latter. As a result, uncontrolled or persistent activation of the AhR by exogenous small molecules may disturb the tightly controlled and transient AhR-regulated cell functions. This could be the underlying cause of the toxicity of

ABBREVIATIONS: AD, atopic dermatitis; AhR, aryl hydrocarbon receptor; AhRE, aryl hydrocarbon receptor responsive element; ARNT, aryl hydrocarbon receptor nuclear translocator; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; DETC, dendritic epidermal cell; ILC, innate lymphoid cell; IEL, intraepithelial lymphocyte; IL, interleukin; IEC, intestinal epithelial cell; IEC, intestinal epithelial cell; IEL, intraepithelial lymphocyte; IL, interleukin; ILC, innate lymphoid cell; ITE, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester; LBD, ligand binding domain; LC, Langerhans cell; MG132, carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; NK, natural killer; PAH, polycyclic aromatic hydrocarbon; PHTH, polyhalogenated aromatic hydrocarbon; SAHRM, selective aryl hydrocarbon receptor modulator; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin ("dioxin"); TCR, T-cell receptor; Th, helper T cell; Treg, regulatory T cell; VAF347, (4-(3-chloro-phenyl)-pyrimidin-2-yl)/(4-trifluoromethyl-phenyl)-amine.
ligands such as dioxins and biphenyls. The link between environment and immunity is particularly intriguing (Kimura et al., 2008; Quintana et al., 2008; Veldhoen et al., 2008; Esser et al., 2009) because it was known that allergies, autoimmune diseases, or immunotoxicity could be caused by chemicals and drugs, although the underlying molecular mechanism and signaling pathways were often unclear. Notably, xenobiotic ligands of the AhR, especially TCDD, are known to adversely affect immune cells, which could simply reflect a disruption of endogenous, AhR-driven homeostasis.

In any case, the strong flexibility of AhR activation by chemicals makes it an attractive pharmacological target, as evident from experiments on stem cell proliferation and inhibition of cancer cells (Safe and McDougal, 2002; Lawrence et al., 2008; Singh et al., 2009; Boitano et al., 2010).

Barrier organs—skin, gut, lung, and eyes, and oral and genital mucosal tissues—specialize in discerning harm from good and can trigger or orchestrate adaptive physiologic responses. Chemicals from the environment, including small molecules from symbiotic and pathogenic microbes, are first encountered at the barriers. With respect to immunologic functions, a network of tissue-specific immune cells is on watch constantly and attracts immune cells from the blood stream or lymph when needed. An important feature of barrier immunity is the distinction between harmful pathogens and harmless microorganisms and protein antigens (e.g., from food), against which active tolerance must be maintained. Moreover, we begin to understand that the (symbiotic) microflora and their metabolic products, in turn, shape immunity and tolerance (Schulz et al., 2011; Mavrommatis et al., 2013). Recently, it was discovered that AhR can be involved in disease tolerance and can also be a sensor of bacterial danger by sensing bacterial pigmented virulence factors (Bessede et al., 2014; Moura-Alves et al., 2014).

High levels of AhR expression were detected in hematopoietic stem cells, in cells of the innate immune system, and also in T-cell subsets and B cells. Exposure to AhR ligands changes the function and fate of these cells. Research using various ligands and/or genetically engineered mouse models has demonstrated that AhR is involved in both correct development of naive cells and in effector functions during an immune response. Here, we review the functions of ligand-activated AhR signaling in the specialized context of barrier organs, especially regarding the immunologic barrier against the environment.

II. Aryl Hydrocarbon Receptor Signaling
A. The Canonical Signaling Pathway

The AhR is a basic helix-loop-helix, Per-ARNT-Sim–containing ligand-dependent transcription factor. It has close structural homology to proteins found in pathways that regulate hypoxia and circadian rhythms (McIntosh et al., 2010). In the absence of ligand, AhR resides in the cytoplasm as a component of a chaperone complex that includes a dimer of Hsp90, together with the cochaperones p23, and AIP, the AhR-interacting protein also known as ARA9, and XAP2 (reviewed by Denison et al., 2011). As depicted in Fig. 1, the translocation of AhR into the nucleus is initiated upon ligand binding and phosphorylation of two protein kinase C sites adjacent to the nuclear localization sequences (Ikuta et al., 2004; McIntosh et al., 2010). AhR dissociates from its chaperone complex and forms a heterodimer with ARNT in the nucleus. The AhR–ARNT dimer then binds to upstream regulatory regions of its target genes (e.g., the cytochrome P450 family 1 gene, CYP1A1). The target genes contain canonical aryl hydrocarbon receptor responsive elements (AhREs, also known as dioxin responsive element or xenobiotic responsive element) having the core sequence 5'-TNCGGTG-3'. The complex with DNA then recruits coactivators, which alter the chromatin structure into a more accessible configuration through histone acetyltransferase and histone methyltransferase activities. Other factors are recruited, including the kinases IKKα, MSK1, and MSK2, the coactivators SP1, NCOA1, NCOA3, NCOA4, and p300, the BRCA1 tumor suppressor protein, and the general transcription factor IIB. The latter is required for transcriptional initiation by RNA polymerase II (Beischlag et al., 2008; Sartor et al., 2009; Taylor et al., 2009; McIntosh et al., 2010; Kurita et al., 2014).

B. Noncanonical Signaling

Cross-talk between the AhR and other signaling pathways can lead to noncanonical mechanisms of actions of the AhR and AhR ligands. Several types of cross-talk have been described as a result of molecular interaction between activated AhR and other proteins, by competition for transcriptional coactivators/repressors, by coactivator-like interactions, or by direct binding (reviewed by Denison et al., 2011). In the nucleus, AhR was found to associate with the hypophosphorylated form of pRB resulting in growth arrest at the G1/S phase of the cell cycle (Levine-Fridman et al., 2004). AhR binds to the transcription factor c-Maf, which is important for regulatory type 1 T-cell differentiation (Apetoh et al., 2010). The AhR also binds STAT1, resulting in nuclear factor-κB promoter activity (Kimura et al., 2009). In addition, interactions of AhR have been described for the estrogen receptor, β-catenin, Nr2, RelA, and RelB (Kim et al., 2000; Vogel et al., 2004, 2007; Miao et al., 2005; Braeuning et al., 2011; Procházková et al., 2011).

Ligand binding to AhR also gives rise to alternative actions (nongenomic) such as a rapid increase in intracellular Ca²⁺ concentration leading to downstream proinflammatory responses mediated by c-src, COX2, and CCL1 (Nebert et al., 1993; NDiaye et al., 2006; Fritsche et al., 2007; Matsumura, 2009; Zhou et al., 2013). Many of the details and the physiologic importance are still unclear.
AhR activity can be modulated by the activity of other signaling pathways. A relevant example is the Wnt/β-catenin pathway. This pathway is important for the maintenance of cell pools through self-renewal of hematopoietic stem cells, intestinal and gastric stem cells, hair and melanocyte stem cells, as well as neural and liver progenitor cells (Clevers, 2006; Tan et al., 2006; Van Camp et al., 2014). It is also essential for development of early T precursor cells, both for the events needed to generate mature naïve T cells and for the peripheral differentiation of naïve T cells into inflammatory and regulatory T-cell subsets (Clevers, 2006; Ma et al., 2012; Xue and Zhao, 2012).

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Of great scientific interest is the role that the AhR seems to play in progenitor cell expansion and differentiation in which the Wnt/β-catenin pathway is active (Laiosa et al., 2003; Singh et al., 2009; Boitano et al., 2010; Latchney et al., 2011; Procházková et al., 2011). Furthermore, a role for AhR in tissue regeneration has been described in models of liver, fin, and cardiac tissue regeneration (Mathew et al., 2006; Mitchell et al., 2006; Hofsteen et al., 2013).

There is an increased understanding of different types of cross-talk between the AhR and Wnt/β-catenin pathways. The Wnt pathway leads to nuclear localization of β-catenin, which can then interact with the DNA-binding TCF/LEF proteins to activate transcription of Wnt/β-catenin target genes. Signaling through β-catenin has been found to work cooperatively with AhR signaling in vitro and in vivo. Both basal and TCDD-stimulated CYP1A1 expression are influenced by the presence of β-catenin (Braeuning et al., 2011). Wnt/β-catenin signaling is involved in both stem cell and cancer cell maintenance and growth. It is tightly controlled by the so-called destruction complex, and either too much or too little β-catenin has negative consequences (Duncan et al., 2005). Notably, the AhR acts as an adaptor protein in the CUL4B:AhR cullin 4B ubiquitin ligase complex (Ohtake et al., 2007). By activating the ubiquitin-proteasome system, the ligand-activated AhR targets several proteins for degradation including the AhR itself, β-catenin, and some sex steroid receptors (Kawajiri et al., 2009; Ohtake et al., 2011).

Consistently, rapidly renewing tissues and cells such as skin, gut, and blood cells are all targets of TCDD
toxicity (as detailed below in section III) and are affected in AhR knockout and AhR-overexpressing mice. Increased knowledge on the interaction of AhR with the Wnt/β-catenin pathway is important to increase our understanding of the role of the AhR in toxicology as well as for the development of new therapeutic applications.

C. Aryl Hydrocarbon Receptor–Dependent Transcription

The AhR transcriptional induction profile has been extensively studied. Binding to the AhRE consensus sequences initiates transcription of a great number of genes. Originally, Daniel Nebert described a battery of six AhR-dependent genes coding for xenobiotic metabolizing enzymes (Nebert, 1989). Today, the number of genes that respond to AhR activation by TCDD or other exogenous compounds is proposed to be on the order of 600 (Sartor et al., 2009; Dëre et al., 2011). Among the most highly upregulated genes is the AhR repressor, which shares structural homology with AhR and ARNT (Mimura et al., 1999), and TIPARP (MacPherson et al., 2014). Both proteins repress AhR. In addition, the genes coding for the CYP1A and CYP1A2 enzymes, which contain multiple functional AhREs in their shared enhancer region, is highly inducible. CYP1 enzymes can metabolically degrade small endogenous high-affinity ligands and can thereby control constitutive AhR-dependent transcription.

Transcriptomics and functional analyses, performed in the presence or absence of exogenous ligands, have revealed AhR target gene networks that control a broad spectrum of cellular functions. This concerns inter alia the immune system and nervous system development, embryonic development, eye development, tube morphogenesis, angiogenesis, and patterning of blood vessels. In the comprehensive genome-wide analysis of the AhR target gene profile performed by Alvaro Puga and coworkers, an unexpected large number of gene promoter regions exhibited significant AhR binding in unstimulated cells (Sartor et al., 2009). They reported that their gene ontology analyses gave unanticipated results that suggested a prominent role of the AhR in regulatory interactions with the Wnt/β pathway as also discussed above (Sartor et al., 2009).

D. The Realm of Aryl Hydrocarbon Receptor Ligands: Agonists and Antagonists

The enigma of an endogenous or physiologic ligand has intrigued the AhR research community for a long time. A three-dimensional structure of the AhR or at least its ligand binding domain (LBD) is still lacking. However, the approximate dimensions of the ligand binding pocket and several critical amino acid residues in the binding pocket are known. This knowledge has enabled docking studies of proposed AhR ligands (Goryo et al., 2007; Soshilov and Denison, 2014). By now, there are only a few types of molecules that have been demonstrated to fit exceptionally well into the LBD and to activate the receptor at concentrations in the picomolar or nanomolar range. Among the high-affinity exogenous substances are several planar and lipophilic PHAHs of the dioxin, dibenzofuran, biphenyl, and azoxybenzene types sharing structural properties with the highly toxic TCDD (Nguyen and Bradfield, 2008). In addition, some planar, lipophilic nonhalogenated PAHs bind to the AhR with high affinity. These include benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3,c,d]pyrene, 3-methylcholanthrene, and β-napthoflavone (Till et al., 1999; Nguyen and Bradfield, 2008). The bacterial pigment 1-hydroxyphenazine and three pharmacological agents, StemRegenin 1 and GNF351 [N-(2-(3H-indol-3-yl)ethyl)-9-isopropyl-2-(5-methyl-3-pyridyl)-7H-purin-6-amine] (two potent AhR antagonists), as well as VAF347 [(4-(3-chloro-phenyl)-pyrimidin-2-yl)-(4-trifluoromethyl-phenyl)-amine], have also been observed to be high-affinity ligands (Lawrence et al., 2008; Boitano et al., 2010; Smith et al., 2011; Moura-Alves et al., 2014). Many of these exogenous compounds probably bear structural similarities to physiologic ligand(s).

The search for bona fide endogenous ligands that induce AhR signaling under physiologic conditions has only produced a small number of candidates. These include one metabolite of arachidonic acid, lipoxin 4A (Schaldach et al., 1999), and the four indoles, FICZ (6-formylindol[3,2-b]carbazole) (Rannug et al., 1987), indirubin (Adachi et al., 2001), ITE [2-(1H-indole-3-carboxyl)-thiazole-4-carboxylic acid methyl ester] (Song et al., 2002), and ICZ (indol[3,2-b]carbazole) (Bjeldanes et al., 1991). In addition, the indolic AhR prolignands such as indole-3-carbinol (I3C) and tryptamine may be converted in the body to the high-affinity ligands ICZ or FICZ (Bjeldanes et al., 1991; Vikström Bergander et al., 2012). L-Kynurenine, kynurenic acid, and cinnaric acid are other endogenous molecules that are formed via catalytic breakdown of tryptophan. They can activate AhR signaling at levels that are physiologically or pathophysiologically relevant (DiNatale et al., 2010; Opitz et al., 2011; DeGroot and Denison, 2014; Lowe et al., 2014).

Table 1 lists different types of small molecules and other factors that have been identified as AhR activators and ligands. In Fig. 2, we display three-dimensional models of high- and low-affinity ligands and an antagonist. Often identification of a chemical as a ligand is accomplished by assays using AhR-dependent transcription and electrophoretic band shifts as proxy readouts. In a note of caution, such assays may not always reflect AhR ligand binding in the LBD. In particular, oxidative stress, inflammatory mediators, and many physical stress factors (exposures that strongly suppress CYP1A1 transcription if added together with inducers such as TCDD or
β-naphthoflavone) have repeatedly been demonstrated to activate AhR-dependent transcription by themselves without direct binding (Gonder et al., 1985; Crawford et al., 1997; Tanaka et al., 2005; Becker et al., 2006; McMillan and Bradfield, 2007; Afacq et al., 2009; Anwar-Mohamed et al., 2009; Wincent et al., 2012). Electrophoretic band shifts exhibiting formation of AhR-AhRE complexes have been detected with nuclear extracts from cells cultured in the absence of added ligands and in cells subjected to various stressful conditions (e.g., hyperoxia, metals, low temperature, immune cell activators, the proteolysis inhibitor MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal), or detached growth (Sadek and Allen-Hoffmann, 1994; Crawford et al., 1997; Monk et al., 2001; Santiago-Josefat et al., 2001; Tamaki et al., 2005; Elbeckai and El-Kadi, 2007). Some authors have suggested the possibility that AhR-DNA interactions might result from disruption of the molecular interaction between the receptor and the chaperone HSP90, through transient increases in AhR or ARNT expression, or through increased production of endogenous ligands (Pongratz et al., 1992; Sadek and Allen-Hoffmann, 1994; Monk et al., 2001; Santiago-Josefat et al., 2001; Kann et al., 2005; Tamaki et al., 2005; Elbeckai and El-Kadi, 2007).

There are two other mechanisms that are primarily discussed with regard to AhR activation by factors that do not bind at all or that do not fit well into the AhR LBD (discussed further in section V). The first one claims that the AhR is promiscuous and AhR signaling can be activated by structurally very diverse chemicals even at low affinity (Denison et al., 2011; Shoshilov and Denison, 2014). The second one claims that seemingly genuine AhR activators inhibit the transcription and/or activity of CYP1A1 and thereby inhibit the metabolic degradation of the endogenous ligand FICZ (Wincent et al., 2012). Shoshilov and Denison (2014) have challenged this latter indirect mechanism. They employed site-directed mutagenesis of the LBD of AhR and describe one particular mutant, I319K, which was activated only by FICZ in a luciferase reporter system. They argue that indirect activation via FICZ, by some other AhR activators tested, could not be involved because these other substances did not activate the I319K mutant. However, FICZ-mediated activation of the I319K mutant was only demonstrated at a relatively high concentration of FICZ (0.1 μM) and not at the picomolar concentrations present in cell culture media under normal cell culture conditions (Oberg et al., 2005; Wincent et al., 2009). Therefore, indirect activation via FICZ cannot be ruled out on the basis of observations with this mutant.

### III. Role of the Aryl Hydrocarbon Receptor in the Barrier Immune System

#### A. Differential and Variable Aryl Hydrocarbon Receptor Expression Levels in Cells and Tissues, Including the Immune System

AhR expression differs significantly between tissues. It is not or only very weakly expressed in muscle tissues, testes, kidney, and brain (both in human and in mouse) (Dolwick et al., 1993; Li et al., 1994; Frericks et al., 2007; Veldhoen et al., 2008). Of course, it is possible that some subsets of cells in these tissues express AhR at high levels, which is not detected when analyzing the whole tissue. Unfortunately, a careful analysis of distinct cell subsets is not available for many tissues. However, constitutive AhR expression is consistently high in liver and in the barrier tissues such as skin, lung, gut, and mucosal epithelia as well as in the placenta. These tissues contain immune cells, many of which express AhR at high levels. With respect to the adaptive immune system, AhR is low in naïve T cells, helper T cells Th1 and Th2, and regulatory T cells but is high in Th17 cells and both the interleukin (IL)-17/IL-22–producing and IL-17/IL-22–nonproducing subsets of peripheral γδ T cells (Veldhoen et al., 2008; Martin et al., 2009). AhR is present at low levels in naïve B cells from the spleen and is induced upon polyclonal activation (Marcus et al., 1998). As reviewed elsewhere, T and B cells are targets of AhR-activating factors. For instance, Th17 cells need AhR activation for IL-22 secretion, and the immunoglobulin locus can be suppressed by TCDD in an AhR-dependent fashion (Esser et al., 2009; Sulecnic and Kaminski, 2011; Quintana and Sherr, 2013). Natural killer (NK) cells express AhR at moderate levels, and AhR activation stimulates antitumor activity as well as resistance to infections (Wagage et al., 2014). However, the examples of naïve B cells or NK cells suggest caution on simplifying projections such as, “AhR is only relevant when expressed in higher amounts.” AhR can be induced by immunologic

<table>
<thead>
<tr>
<th>AhR Activators with No or Very Low Binding Potential</th>
<th>Low-Affinity Ligands (Binding Affinity in the Micromolar to Millimolar Range)</th>
<th>High-Affinity Ligands (Binding Affinity in the Picomolar to Nanomolar Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory mediators, glutathione depletion, hydrogen peroxide, hyperoxia, metals and metalloids, neurotoxins, organic solvents, oxidized lipids, ozone, quartz, shear stress and miscellaneous materials, etc.</td>
<td>Drugs, biochemical model compounds, combustion products, food mutagens, hair dyes, hemes, humic acids, indoles, flame retardants, food additives, phytochemicals, plastic materials and additives, specific antagonists and blockers, etc.</td>
<td>FICZ, GNF351, ICZ, indirubin, ITE, lipoxin 4A, StemRegenin 1, TCDI and some other PHAs, some PAHs, VAF347, and 1-hydroxyphenazine</td>
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stimuli and then become essential for downstream differentiation events. There are still many unknowns, which need to be solved before starting therapeutic manipulation of the receptor in a cell-specific manner. A summary of the current knowledge regarding AhR expression levels in immune cells is displayed in Fig. 3.

Mouse models with no or modified AhR signaling have been generated and are used in studies of the immune system (Esser, 2009), and data emerge also from newly bred conditional mouse lines in which AhR deficiency is restricted to certain cells (Kiss et al., 2011; Di Meglio et al., 2014). AhR deficiency and AhR dysregulation by xenobiotics have considerable consequences for barrier tissue development and function of cells of the adaptive and innate immune system. For example, mice with a constitutively active AhR in keratinocytes are afflicted with inflammatory skin diseases (Tauchi et al., 2013). Together, the findings argue strongly for the view that AhR is an evolutionary shaped, signaling pathway for physiologic immune functions. In the immune system, it balances and integrates environmental cues into an appropriate immune response.

B. Aryl Hydrocarbon Receptor in the Skin

The skin offers protection from environmental stresses such as dehydration, UV light, mechanical trauma, and infections. The skin is a layered organ, with the epidermis on top, the much thicker dermis underneath, and finally the subcutis as the innermost layer. Blood and lymphatic vessels reach into the dermis and serve as ports for emigrating and immigrating immune cells. Resident immune cells are interspersed in both the dermis and epidermis.

1. The Dermis

The dermis has high cell diversity; the structural cells are the fibroblasts, which secrete matrix proteins and hyaluronic acid. Interspersed among the fibroblasts are macrophages, mast cells, dendritic cells (DCs), and many T cells, all with differing levels of AhR expression (Esser et al., 2013). For humans, it has been estimated that more T cells are in the skin than in the blood (Clark et al., 2006). Dermal fibroblasts express the AhR at high levels. Interestingly, they upregulate matrix metalloproteinase-1 but do not upregulate CYP1A1 upon AhR ligand exposure. The former result relates to the role of AhR in UV-mediated skin aging (Ono et al., 2013; Tigges et al., 2014), whereas the latter has been interpreted as a protection against generation of reactive oxygen species in these mostly postreplicative cells.

2. The Epidermis

The structural cells of the epidermis are the keratinocytes, which differentiate from the innermost stem cell layer to the outer stratum corneum layer, with enucleated corneocytes forming a water-tight final barrier. Keratinocytes gain AhR and CYP1A1 expression along this "outward" differentiation (Jones and Reiners, 1997; Swanson, 2004). Interspersed in the epidermis are approximately 5% Langerhans cells (LCs), a subset of DCs) and, in mice, also approximately 5% dendritic epidermal T cells (DETCs, with a skin-typical, invariant γδ T-cell receptor (TCR) for which the antigen specificity is not known). Together with the keratinocytes, they form an immune network with surveillance capacities (Jameson et al., 2004). Similar to dermal fibroblasts, keratinocytes express AhR; LCs, DETCs, melanocytes, sebocytes, and mast cells also express AhR (Esser et al., 2013), as do tissue-resident CD8+ memory T cells (Zaid et al., 2014). Interestingly, a concomitant high constitutive expression of AhR repressor has been reported for LCs, DETCs, and fibroblasts (Haarmann-Stemmann et al., 2007; Jux et al., 2009; Tigges et al., 2013).

3. Aryl Hydrocarbon Receptor–Mediated Skin Functions

It is commonly assumed that the high AhR levels in all skin cell types serve some physiologic purpose (Ma, 2011). The search for such physiologic role(s) of AhR in the epidermis is ongoing, using AhR overactivation on the one hand and AhR-deficient mouse models on the other hand. Studies involving AhR-deficient mice indicated that AhR is needed for proliferation of melanocytes and thus pigmentation (Jux et al., 2011). In humans, epidemiologic studies strongly suggested that air pollution from soot and traffic (which contains AhR ligands) is correlated to increased extrinsic skin aging (Vierkötter et al., 2010). Studies with topical or systemic application of AhR ligands (e.g., TCDD or FICZ) demonstrated that AhR signaling is involved in degranulation and cytokine production of mast cells (Sibilano et al., 2012) and differentiation of sebocytes (Ju et al., 2011). In keratinocytes, AhR is involved in the UVB stress response (Fritzsche et al., 2007) and antiapoptotic signaling in response to UV (Frauenstein et al., 2013). AhR presence in skin can protect against UV-induced erythema or change the gene expression profile of keratinocytes. Moreover, epithelial-to-mesenchymal transition and motility of keratinocytes is enhanced in AhR-deficient keratinocytes. Finally, AhR is involved in UV-induced immunosuppression (Navid et al., 2013; Rico-Leo et al., 2013; Bruhs et al., 2015).
4. Aryl Hydrocarbon Receptor in Two Skin-Specific Innate Immune Cells. DCs phagocytize antigens and process and present them to T cells. In addition, they secrete cytokines and factors, which provide a proinflammatory or immunosuppressive milieu, shaping the differentiation pattern of T cells into inflammatory or regulatory subsets. LCs are the resident DCs in the epidermis. In contrast with dermal DCs, they presumably are tolerogenic by default (Kaplan et al., 2008). LCs continuously sample skin antigens and migrate to the nearest lymph node to present their antigen to T cells. Studies in AhR-deficient mice indicated that AhR is necessary for the maturation of LCs and their antigen-presenting capacity (Jux et al., 2009). Expression of maturation markers could be rescued by GMCSF, a cytokine also produced by DETCs, which are lacking in AhR-deficient mice, as detailed below. As a consequence, AhR-deficient mice do not mount a strong contact hypersensitivity response when challenged with chemical haptens, such as fluorescent isothiocyanate or dinitrofluorobenzene.

DETCs are unconventional immune cells, with an invariant γδ TCR. As with all epithelial invariant γδ T cells, they are generated exclusively in a tight time window in the fetal thymus. γδ T cells secrete inflammatory cytokines quickly after antigen contact and are pivotal in fighting bacterial infections and killing tumor cells (Hayday et al., 1985; Girardi et al., 2002; Strid et al., 2009). They are an important source of IL-17 and IL-22, and thereby are also involved in autoimmunity (Roark et al., 2008). DETCs are abundant in the murine epidermis but not in the human epidermis. However, humans have a similar population of γδ T cells in their dermis (Holtmeier and Kabelitz, 2005). Apparently, human dermal γδ T cells recognize stress-inducible molecules, such
as MICAB, as well as other ligands. In mice, DETCs play an important role in the control of inflammatory skin reactions, in wound healing, and in cancer surveillance (Strid et al., 2009). DETCs immigrate into skin shortly before birth and expand by proliferation within a few weeks thereafter. This expansion does not take place in AhR-deficient mice. Their skin niche remains empty (in contrast before birth and expand by proliferation within a few weeks (Strid et al., 2009). DETCs immigrate into skin shortly

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in the Skin.

AhR antagonists to protect from UVB-associated skin

be exploited pharmacologically. Examples are the use

of medical coal tar described above as well as the use of

barrier impairment. As indicated for the human epidermis, activated AhR induces production of pivotal barrier proteins by keratinocytes and accelerates epidermal barrier formation in mouse fetuses (Sutter et al., 2011). Accordingly, we have observed increased transdermal water loss in AhR-deficient mice, congruent with barrier impairment (C. Esser, unpublished data). Interestingly, an old and efficient therapy for atopic dermatitis (AD) is treatment with medical coal tar, a substance rich in AhR ligands. Barrier impairment is a prominent feature of AD. It was demonstrated in organotypic skin cultures from AD patients that coal tar activated the AhR, induced epidermal differentiation, restored filaggrin expression, and improved skin barrier proteins in an AhR-dependent manner (van den Bogaard et al., 2013).

In conclusion, the skin is a site of high AhR expression and high AhR ligand availability, and the AhR is involved in many skin functions, including the skin immune network and cell homeostasis. This can be exploited pharmacologically. Examples are the use of medical coal tar described above as well as the use of AhR antagonists to protect from UVB-associated skin cancer (Tigges et al., 2014).

5. High-Affinity Aryl Hydrocarbon Receptor Ligands in the Skin. It is intriguing to think of skin-located, abundant AhR ligands as evolutionary drivers of these functions. In the skin, AhR ligands are formed in situ by several sources. UV light generates the high-affinity ligand FICZ from tryptophan. In addition, hydrogen peroxide in skin of vitiligo patients can lead to formation of FICZ (Schallreuter et al., 2012). Common skin-resident yeasts, Malassezia spp., produce the AhR ligands FICZ, ICZ, pityriacitrin, and malassezin (Wille et al., 2001; Gaitanis et al., 2008; Magiatis et al., 2013). LCs produce AhR-dependently the immunosuppressive enzyme indoleamine 2,3-dioxygenase (Jux et al., 2009; Nguyen et al., 2010), which converts tryptophan to kynurenines. Kynurenines as AhR ligands thus enhance their own production, generating an immunosuppressive micromilieu in the skin (Mezrich et al., 2010; Nguyen et al., 2010). Kynurenines can also induce regulatory T cells (Tregs) directly (Mezrich et al., 2010). In addition, AhR activators can be present in topically applied cosmetics, creams, or drugs.

C. Aryl Hydrocarbon Receptor in the Gut and Consequences for Gut Health and Microbiome

1. The Gut-Associated Immune System: A Paradigm of Immune Activation and Suppression. The gut is not only in contact with dietary compounds but is also a site of contact with numerous organic and inorganic environmental compounds. The small and large intestines harbor a highly diverse microflora; in humans, it is estimated that approximately $10^{14}$ bacteria live in the gut. Many of them are from the phyla firmicutes (65%) and bacteroidetes (30%) (Qin et al., 2010). Of note, food—its proteins, carbohydrates, and lipids—is a rich source of potential antigens, and immune responses against food constituents can lead to allergies and intestinal inflammation. It is thus vital that the gut-associated immune system protects against infection while it maintains tolerance against harmless antigens. The gut-associated immune cells specialize in this dual task. The gut content is separated from the inner body only by a single epithelial cell layer of intestinal epithelial cells (IECs) and a mucus layer secreted by goblet cells. The gut epithelium allows passage of nutrients from the lumen into the blood stream, and the mucus has important functions in protecting the integrity of the epithelium. Interspersed among the IECs are intraepithelial lymphocytes (IELs), many of them invariant γδ T cells, others αβTCRCD8α+ T cells. Underneath the epithelium, in the lamina propria, more immune cells reside, especially DC subpopulations and innate lymphoid cells (ILCs). Breakage of the intestinal barrier results in potentially deadly inflammation and sepsis.

2. Aryl Hydrocarbon Receptor in Innate Immune Cells of the Gut. Excitingly, a reciprocal relationship between gut bacteria and their metabolites and the gut immune system exists, in which the AhR also plays a role. AhR-deficient RORγT+ ILCs (a major source of intestinal IL-22) have reduced IL-22 expression; therefore, AhR-deficient mice easily succumb to Citrobacter rodentium infection (Qiu et al., 2013). Qiu et al. (2013) indicated that treatment with FICZ (0.5 μg/kg) significantly increased the accumulation of RORγT+ ILCs in AhR−/− or AhR+/+ mice but not in AhR−/− mice. In another study, Zelante et al. (2013) analyzed lactobacillus species (nonpathogenic gut bacteria) and presented evidence that these bacilli can generate AhR ligands in the gut, such as indole-3-aldehyde, from tryptophan and thereby enhance AhR-dependent IL-22 production. In a reporter assay, indole-3-aldehyde induced AhR-dependent
transcription but only at high concentration, suggesting that it is a low-affinity ligand. However, indole-3-acetaldehyde, one of the products formed together with indole-3-aldehyde, can produce the high-affinity ligand FICZ (Rannug et al., unpublished data), and this could be relevant for the effects observed by Zelante and colleagues.

IL-22 acts on epithelial cells and induces their production of antimicrobial peptides (e.g., RegIIIγ) and stimulates tissue regeneration. As a result, commensal bacteria might outcompete pathogenic bacteria and prevent colonization with the fungus *Candida albicans* (Zelante et al., 2013). Similar to the situation in skin keratinocytes and skin immune cells, the AhR is expressed highly in IECs and in cells of the gut-associated immune system. AhR-deficient mice have highly reduced IEL numbers in the small intestine (Chmill et al., 2010; Li et al., 2011; Nakajima et al., 2013), which is associated with reduced levels of IL-22 and thus a reduction of the antimicrobial peptides RegIIIβ and RegIIIγ (measured in the ileum) and a higher microbial load in both the small intestine and colon (Li et al., 2011). The loss of IEL is cell intrinsic, because AhR-deficient bone marrow cells did not reconstitute the intestine in Rag−/− mice (Li et al., 2011). Moreover, in the gut of AhR-deficient mice, subsets of ILC3s (Spits et al., 2013), ILC22 and CD3−NKp46+ lymphoid tissue inducer cells, are lost over time after birth (Kiss et al., 2011; Lee et al., 2012). Again, failure of ILC3 to proliferate in AhR-deficient mice is an intrinsic effect, because the AhR is needed to transcribe a cell-specific proliferation factor, c-kit (Kiss et al., 2011; Lee et al., 2012). As a consequence, no secondary lymphoid structures, such as cryptopatches or innate lymphoid follicles, are formed in the intestine of AhR-deficient mice, and they become susceptible to *C. rodentium* infection (a model for attaching and effacing *Escherichia coli* EHEC in humans). ILC3s are characterized by IL-17 and IL-22 secretion (Diefenbach, 2013; Spits et al., 2013). AhR-deficient mice are not only more susceptible to *C. rodentium* infection, they are also more susceptible to dextran sodium sulfate (DSS)–induced murine colitis. DSS damages gut epithelial integrity and causes inflammation and bacterial dissemination. AhR-deficient mice constituted with wild-type IELs did not succumb to DSS colitis, demonstrating the importance of IELs in reducing the damage (Lee et al., 2012).

3. Systemic Effects of Oral Aryl Hydrocarbon Receptor Ligand Exposure. The role of the AhR on cells of the adaptive immune system, especially on T cells, was also reviewed in *Pharmacological Reviews* (Quintana and Sherr, 2013). As reviewed there, mice generate protective Tregs if treated orally with the AhR agonist ITE (originally isolated from swine lung) before subjecting them to a protocol of immunization with myelin oligodendrocyte glycoprotein MOG35–55 to induce experimental allergic encephalitis (Gandhi et al., 2010; Quintana et al., 2010). The authors concluded that ITE mediates the effect by inducing CD103+ DCs in the gut, which then support Treg generation. In another study, however, it was found that TCDD exposure of mice impaired the capacity to establish stable oral tolerance against a food antigen (Chmill et al., 2010), presumably by inducing an inflammatory state with higher levels of IL-6 secretion in gut-derived DCs. It is possible and likely that AhR ligands as such (affinities, degradability, etc.) as well as the exposure regimens are decisive in the outcome for the tolerogenicity versus immunogenicity balance in the gut (Duarte et al., 2013).

4. Role of Dietary Ligands in Development of the Innate Gut Immunity. Dietary AhR ligands are necessary for constitutive CYP1A1 levels and induction in the gut (Ito et al., 2007). An intriguing set of experiments demonstrated the role of dietary AhR ligands for innate immune cell homeostasis in the gut. Experimental mouse diets are for the most part grain based and contain high concentrations (up to several grams per kilogram) of AhR activators, such as polyphenols and glucosinolates. Colonies of mice were fed with a synthetic diet of very low AhR activator content, and then newborn mice were analyzed 3 to 4 weeks after birth. Mice had a similar phenotype as AhR-deficient mice: low-level c-kit expression, low ILC3 numbers, and because of ILC3 phenotype (Li et al., 2011). It is not known what the specific proliferation factor, c-kit (Kiss et al., 2011; Lee et al., 2012). As a consequence, no secondary lymphoid structures, such as cryptopatches or innate lymphoid follicles, are formed in the intestine of AhR-deficient mice, and they become susceptible to *C. rodentium* infection (a model for attaching and effacing *Escherichia coli* EHEC in humans). ILC3s are characterized by IL-17 and IL-22 secretion (Diefenbach, 2013; Spits et al., 2013). AhR-deficient mice are not only more susceptible to *C. rodentium* infection, they are also more susceptible to dextran sodium sulfate (DSS)–induced murine colitis. DSS damages gut epithelial integrity and causes inflammation and bacterial dissemination. AhR-deficient mice constituted with wild-type IELs did not succumb to DSS colitis, demonstrating the importance of IELs in reducing the damage (Lee et al., 2012).

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suggests that AhR activation in the gut by environmental pollutants or possibly even food additives can have both adverse and beneficial effects on oral tolerance.

Both findings—the importance of AhR signaling for the development of innate immune cells and for oral tolerance—have potential therapeutic applications. Plant-derived compounds may be used in the future to therapeutically address diseases such as necrotizing enterocolitis of preterm infants (Neu and Walker, 2011) or inflammatory diseases of the gut. In addition, enhancement of oral tolerance might be useful in therapies whereby autoantigen is given orally to dampen autoimmunity (Thurau et al., 2004; Yeste et al., 2012).

**D. Aryl Hydrocarbon Receptor in the Lung**

Lung epithelial tissue, similar to the gut, is a mono-layer of cells. Several subsets, including the basal stem cells, the ciliated cells, the goblet cells (for mucus production), and the brush cells, line the airways; the epithelium and its mucus layer become thinner toward the alveoli. Underneath the epithelium is a basal matrix in which immune cells are found (mast cells, lymphocytes, DCs, and ILC groups 1, 2, and 3). Lung tissue expresses the AhR at high levels (Li et al., 1994; Frericks et al., 2007).

The lung is exposed to AhR activators present in airborne particulate matter from charcoal or wood burning, industrial exhausts, car emissions, cigarette smoke, or urban dust. PAHs are often part of air pollution particulate matter and have existed as environmental agents long before industrialization. Particulate matter has different effects depending on chemical composition as well as size, with nanoparticles (defined as 1–100 nm in diameter) even capable of entering cells and the nucleus (Hemmerich and von Mikecz, 2013). AhR expression by lung epithelium allows detection and metabolic elimination of unwanted xenobiotic pollutants through induction of CYP1 enzymes. Another (mechanical) possibility is the regulation of mucus secretion; the genes for MUC5B and MUC5AC could be targeted by the activated AhR in a lung epithelial cell line (Wong et al., 2010; Chiba et al., 2011). In vivo mucus production enforces the physical protection of the lung epithelium. In another study, the AhR was demonstrated to protect fibroblasts from apoptosis caused by cigarette smoke (Rico de Souza et al., 2011). At the same time, AhR activity could be an integral part of a balanced immune response. It can, again that the AhR is used in a barrier organ to orchestrate an appropriate immune response. It can, however, become unbalanced by persistent and high activation. It is thus no surprise that epidemiologic evidence found that exposure to environmentally derived AhR activators correlates with increased respiratory infections (Del Donno et al., 2002).

Epidemiologic studies have linked exposure to air pollution to immunologic diseases of the respiratory system, such as asthma and chronic obstructive pulmonary disease (COPD), innate immune cells, and polymorphisms of inflammatory cytokines (Morgenstern et al., 2008; Vawda et al., 2014; Yu et al., 2014). To link air pollution to these lung diseases, experimental exposures to AhR ligands, AhR-deficient mice, and various infection or lung damage models are used to identify underlying mechanisms and the role of the AhR.

1. **Lung Inflammation.** Lung inflammation can be caused by AhR activators, such as those present in cigarette smoke. Studies in AhR-deficient mice demonstrated that exposure to cigarette smoke for only a few hours on 3 consecutive days before euthanasia enhanced neutrophil influx and levels of inflammatory cytokines IL-6, macrophage inflammatory protein 2, and prostaglandin E2 and increased tissue damage (Thatcher et al., 2007). The contribution of innate versus adaptive immune cells to lung pathology is still under investigation, and many inflammatory mediators have been implicated as well. Macrophages, cells critical for the development of inflammatory processes, express the AhR and can be activated by TCDD to produce modulatory chemokines or release proinflammatory cytokines (Vogel et al., 2007).

2. **Respiratory Viral Disease.** The AhR is a modulator of antiviral immunity in the lung. In particular, the work by the group of Paige Lawrence demonstrated the complex relationship of AhR-mediated events in CD8+ T cells, DCs, neutrophils, and lung epithelium in influenza A virus–infected mice (Vorderstrasse et al., 2003; Jin et al., 2014). AhR activation by TCDD decreased survival from infection with a nonlethal dose of influenza A virus (Vorderstrasse et al., 2003), accompanied by doubled pulmonary neutrophil influx and suppression of expansion and differentiation of virus-specific effector CD8+ T cells (Lawrence et al., 2006). At the same time, AhR activation decreased the immunostimulatory function of DCs in lung-draining lymph nodes and reduced their frequency in the lungs (Wheeler et al., 2013; Jin et al., 2014). Neutrophil influx is mediated by the AhR in the lung epithelium because conditional AhR-deficient mice (lacking the AhR only in lung epithelium) had no increased neutrophil influx upon TCDD exposure. Both TCDD and FICZ could modulate the anti–influenza A response, although FICZ only affected CD8+ cells (Wheeler et al., 2014). Together, the data in mice highlight again that the AhR is used in a barrier organ to orchestrate an appropriate immune response. It can, however, become unbalanced by persistent and high activation. It is thus no surprise that epidemiologic evidence found that exposure to environmentally derived AhR activators correlates with increased respiratory infections (Del Donno et al., 2002).

3. **Lung Fibrosis.** Similar to the skin and the gut, the murine lung harbors a unique population of invariant γδ T cells (Vγ6/Vδ1), which are one source of IL-22 production. Mice with either no γδ T cells or with the low-affinity AhR d-allele mount reduced IL-22 levels in the lung upon *Bacillus subtilis* infection, leading to...
more fibrosis. Administration of IL-22 improves lung inflammation and collagen deposition (Simonian et al., 2010). Together, the data demonstrate that protective IL-22 expression in this inflammation-induced pulmonary fibrosis is AhR dependent. Of note, unlike in the skin or the gut, γδ T cells do not disappear from the lung in AhR-deficient mice (Simonian et al., 2010), indicating that intrinsic proliferation requirements and/or intercellular interactions are different in the lung tissue than in skin and gut.

IV. The Aryl Hydrocarbon Receptor in Toxicology and Physiology of the Human Skin, Lung, and Gut

Species differences in TCDD toxicity and AhR signaling are well known. It is important to understand AhR signaling in human biology, both for toxicology and for any possible therapeutic use of the AhR and its ligands. Research draws on results from epidemiology, accidental environment exposures, tissue culture, and patient studies. In this section, we address some AhR events typical for environment exposures, tissue culture, and patient studies. Research draws on results from epidemiology, accidental any possible therapeutic use of the AhR and its ligands. Signaling in human biology, both for toxicology and for ing are well known. It is important to understand AhR signaling in skin and gut.

In contrast with some animals, in which dioxins can cause death after even tiny doses, dioxin-like compounds are not acutely toxic/deadly in humans even at high exposures, and skin is often the main organ that is affected by PHAHs. Chloracne, hyperpigmentation, and thickening of the skin on palms and soles have been reported after exposure to a number of dioxin-like agents. Chloracne, the hallmark of dioxin toxicity in humans, is characterized by epidermal hyperplasia, disappearance of sebaceous glands, and multiple epithelial cysts, which are now described as hamartomas (Saurat et al., 2012). The same pathologic responses to TCDD, including hyperkeratosis, epidermal hyperplasia, sebaceous gland involvement, and intraepidermal keratinous cysts have earlier been described in mice homozygous for mutations in the hairless gene (hr/h) but not in other naked mice (Puhvel et al., 1982). The hairless protein is now known to be a nuclear receptor corepressor that regulates hair regeneration by interacting with Wnt/β-catenin signaling (Beaudoin et al., 2005). Because the sebaceous gland and hair follicle epithelium have the same cellular source for renewal, chloracne is now suggested to be caused by a switch from a sebaceous to epithelial type of differentiation mediated through transient activation of the nuclear protooncogene c-Myc, which is a target of the Wnt/β-catenin pathway (Panteleyev and Bickers, 2006; Saurat et al., 2012). A recent study with TCDD treatment of human skin samples exposed ex vivo and cultured human SZ95 sebocytes supports this suggestion. The authors show TCDD- and AhR-dependent shrinkage of sebaceous glands with a switch of sebaceous into keratinocyte-like differentiation, eventually by targeting sebaceous progenitor cells (Ju et al., 2011). In addition, hyperpigmentation of human skin and gingiva has been observed after exposure to both PAHs and PHAHs (Urabe and Asahi, 1985; Haresaku et al., 2007; Nakamura et al., 2013). The mechanisms behind AhR-mediated pigmentation were first described by us (Luecke et al., 2010; Jux et al., 2011) and were recently further elucidated by Nakamura et al. (2013). They provide evidence for involvement of both AhR and Wnt/β-catenin signaling in tobacco smoke extract–induced melanocyte activation (Nakamura et al., 2013). In addition, hyperkeratotic skin conditions have been reported in humans exposed to dioxins (Geusau et al., 2000). Correct palmoplantar keratinization depends on intact Wnt/β-catenin signaling, and palmoplantar keratoses are seen in humans that carry mutations in the gene coding for R-spondin, an agonist of Wnt/β-catenin signaling (de Lau et al., 2012).

Induction of the AhR target gene CYP1A1 has been repeatedly documented in human skin and cultured human keratinocytes. For instance, increased benzo[a]pyrene hydroxylation capacity was observed in skin from coal tar–treated patients with dermatological diseases (Bickers and Kappas, 1978). UV light induces CYP1A1 in human skin (Katiyar et al., 2000) and cultured human keratinocytes (Wei et al., 1999; Fritsche et al., 2007). Furthermore, the proposed endogenous AhR ligand FICZ has been detected in UVB-irradiated immortal human keratinocytes (Fritsche et al., 2007), in skin samples from vitiligo patients (Schallreuter et al., 2012), and in skin from patients with seborrheic dermatitis carrying commensal yeasts belonging to the genus Malassezia, which converts tryptophan to several AhR-activating compounds (Magiatis et al., 2013).

There are also systemic manifestations, which have been attributed to the exposure to PHAH in humans exposed in environmental accidents and at the workplace and for human volunteers (Suskind, 1985; Aoki, 2001; Saurat et al., 2012). Chronic bronchitis–like symptoms were observed in over 60% of Yusho patients that had been exposed to cooking oil contaminated with polychlorinated dibenzofuran type (Aoki, 2001). Epidemiologic reports from the 1976 Seveso accident in Italy have associated TCDD exposure with a more than doubled incidence of COPD (Consonni et al., 2008). COPD affects approximately 200 million people worldwide and is globally one of the leading causes of death. The disease starts with inflammation, influx of macrophages and neutrophils, and oxidative stress, which exacerbates inflammation (Roca et al., 2013). Smoking, biomass burning, air pollution, and inhalation of fine dust are causative agents (Brunekreef and Forberg, 2005). Atmospheric particulate matter—known to be carriers of AhR ligands—aggravates COPD. Further understanding the role of the AhR in COPD is an important area for future research (van Voorhis et al., 2013).

Furthermore, nausea, vomiting, gastritis, and colitis with multiple ulcers have been documented in many
epidemiologic studies of PHAH-exposed groups (Kuratsune et al., 1972; Suskind, 1985; Urabe and Asahi, 1985). In addition, the dioxin-poisoned former Ukrainian president Yushchenko initially suffered from severe gastritis (Saurat et al., 2012). A study performed with nonhuman primates fed chlorinated biphenyls and triphenyls indicated hyperplasia and dysplasia of the gastric mucosa with replacement of the gastric acid–secreting parietal cells by mucus-secreting cells (Allen and Norback, 1973). Interestingly, constitutively active AhR causes similar gastric lesions in mice, including gastric hamartomatous tumors (Andersson et al., 2005).

These and other findings indicate important roles of the AhR in rapidly renewing human tissues and demonstrate key roles for interactions with the Wnt/β-catentin pathway, which seem to explain the skin disorders, including chloracne, that occur in humans exposed to TCDD.

A compilation of the facts described in sections III and IV is displayed in Fig. 4.

V. The Aryl Hydrocarbon Receptor: Promiscuous Sensing of Chemicals or Metabolic Control of the Endogenous High-Affinity Ligand FICZ?

From the evidence reported above and many other studies, it is evident that numerous chemicals can interfere with the AhR signaling system. From this, it was concluded that the AhR is a promiscuous sensor of small chemicals; in other words, even low-affinity small chemicals affect physiologic functions through binding and activation. We think that the evidence is not conclusive, first, because the very low affinity of many such putative ligands is not sufficiently taken into account; and second, because the presence of the high-affinity ligand FICZ, omnipresent under cell culture conditions and probably in all tissues, has to be considered in the interpretation of the data. An influence on the metabolic turnover of the endogenous ligands could be a major reason for the dysbalance observed with factors that induce or inhibit the activity of CYP1 enzymes (Fig. 5).

The receptor is highly selective for molecules having certain characteristics constricted by size, lipophilicity, and shape. A view that the AhR has few endogenous ligands is supported by the fact that the AhR is evolutionary conserved and that the protein is present even in the most primitive vertebrate species. This suggests that it has a fundamental role in cellular physiology. In addition, several results from studies with AhR knockout mice have demonstrated functions that need the AhR under developmental stages and for physiologic homeostasis processes without exogenously administered ligands (Gasiewicz et al., 2014).

According to what is now known about FICZ, ICZ, and possibly ITE and other indoles, these molecules seem to fulfill the role of endogenous ligands that maintain important functions in biologic systems (Ma, 2011). In particular, FICZ binds to the AhR with the highest affinity yet reported (Rannug et al., 1987; Nguyen and Bradfield, 2008). FICZ also binds to frog and bird AhRs where TCDD is relatively inactive. This indicates evolutionary conservation of the FICZ response in TCDD-insensitive species, suggesting its physiologic importance as an AhR ligand (Laub et al., 2010). FICZ and ICZ are also efficiently autoregulated by the induced CYP1 enzymes (Wei et al., 2000; Bergander et al., 2004; Wincent et al., 2009). In fact, the catalytic efficiency of CYP1A1 for FICZ is close to the limit of diffusion (Wincent et al., 2009). FICZ has been identified in human skin (Wincent et al., 2009; Schallreuter et al., 2012; Magiatis et al., 2013), and FICZ-derived sulfate conjugates have been detected in human urine (Wincent et al., 2009). The current data suggest that FICZ can be formed via different pathways that lead to formation of indole-3-acetaldehyde, the precursor of FICZ, such as enzymatic deamination of tryptamine and oxidation of tryptophan by intracellular oxidants (Rannug et al., unpublished data). Occurrence of ICZ may be restricted to the gut. It is entirely conceivable that there are more ligands with similar chemical structures that are capable of fulfilling AhR-regulated functions in a tissue-specific manner.

The important role of efficient control over the metabolic turnover of FICZ is illustrated by recent data suggesting that FICZ can stimulate various signaling pathways and thereby affect critical and specific cell functions (see Table 2).

It is now well recognized that unintended, ill-timed, or prolonged activation of the AhR by many exogenous small molecules may lead to expression, synthesis, and activity of many proteins. This in turn may disturb tightly controlled and transient physiologic functions (Mitchell et al., 2006; Stockinger et al., 2014). This seems to explain the toxicity of TCDD, which is unmatched by any other human-made substance. This fact has to be considered when trying to identify pharmacological agents with therapeutic potential by interfering with AhR signaling because they may disturb such tightly controlled and transient signals and lead to unwanted toxicity. The growing knowledge about factors that interfere with AhR signaling and the high specificity and flexibility, however, demonstrates ample possibilities for preventive actions.

VI. Therapeutic Potential of Aryl Hydrocarbon Receptor Ligands

The therapeutic potential of the AhR has been recognized early, especially in the context of cancer (Safe et al., 1999, 2013). The effects of AhR ligands as agonists or antagonists are dependent on several factors including ligand structure, specific gene, and cell context–dependent expression of important cofactors or coactivators. Thus, selective aryl hydrocarbon receptor modulators (SAHRMs) have been developed with a view to clinical applications
For instance, 12 weeks of feeding 6-methyl-1,3,8-trichlorodibenzofuran inhibited metastasis in a mouse model of prostate tumorigenesis, in part by inhibiting prostatic vascular endothelial growth factor production prior to tumor formation (Fritz et al., 2009). SAhRMs aim to modify AhR activation in such a way that the beneficial effects outweigh toxic effects (e.g., those known from dioxins). The complexity of AhR signaling outcomes is evident from the above sections. There are several obstacles. First, an AhR ligand or proligand might be rapidly metabolized, and both the original compound and metabolites may be involved in activities independent of AhR signaling. In particular, this is true for antioxidants, such as resveratrol and quercetin. Other compounds are prone to formation of DNA adducts or protein adducts, which may cause adverse effects. An example of this is glucosinolates, which form DNA adducts (Schumacher et al., 2014). The overall efficiency of a SAhRM will depend on its chemistry and on multiple factors including the dose, pharmacokinetics, absorption, persistence, and other properties that must be considered for development of a new pharmaceutical. For example, poor absorption limits the effectiveness of the AhR ligand, GNP351 (Fang et al., 2014), which exhibited promising anticancer properties in head and neck cancer cells in culture (DiNatale et al., 2012). Yeste et al. (2012) investigated the delivery to the gut of the ligand ITE, when ITE was packed in nanoparticles (Yeste et al., 2012). Indeed, this approach may enhance bioavailability.

Two other sources of AhR ligands with pharmaceutical potential are currently considered. First, various dietary phytochemicals were identified as potential ligands. Phytochemicals are non-nutrient plant compounds, which can be classified into phenolic compounds, terpenoids, alkaloids, phytosterols, and carotenoids. Phytochemicals are absorbed, metabolized, and effluxed into the blood stream by IECs. Biotransformation is catalyzed by enzymes controlled by the AhR battery, the cytochrome P450s and phase II enzymes. For a number of polyphenols, especially the large group of flavonoids, immunomodulation and AhR activation were demonstrated (Denison and Nagy, 2003; González et al., 2011). Second, AhR ligands are generated in cells and can be, for example, products of amino acid metabolism. The tryptophan-derived ligand FICZ is discussed above in detail. Other tryptophan derivatives, the kynurenines, and further downstream products such as the newly identified cinnabarinic acid (stimulates IL-22) display...
large potential as well (Mezrich et al., 2010; Lowe et al., 2014). Although global gene expression profiles and promoter studies have shed some light on the activities of SAhRMs, AhR antagonists and agonists, and selective modulators for other receptors (Sun et al., 2004; Nohara et al., 2006; Suzuki and Nohara, 2007; Nault et al., 2013), the factors important for the selectivity of a specific AhR ligand are not well understood and require further investigation.

One example highlights the complexities of using AhR ligand in therapy. With regard to the immune system, the capacity of AhR agonists to shift the balance between inflammatory, autoimmune-prone Th17 responses and immunosuppressive responses driven by Tregs is of considerable interest. Among the Th cell subsets, only Th17 cells express AhR at high levels. Under in vitro Th17 differentiating culture conditions, AhR ligands (even low amounts of FICZ produced by light from tryptophan present in cell culture medium) promote the generation of the Th17 phenotype, including secretion of IL-17 and IL-22. It was thus unexpected that in mice in vivo, the severity of two crippling autoimmune diseases (experimental allergic encephalitis or experimental allergic arthritis) as well as inflammatory colitis, decreased upon exposure to TCDD, ITE, or FICZ (Quintana et al., 2008; Monteleone et al., 2011; Nakahama et al., 2011). Autoimmunity is driven by Th17 cells and ameliorated or prevented by antigen-specific regulatory T cells. In the

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<th>Biologic End Point</th>
<th>In Vitro References</th>
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<td>Antitumor activity</td>
<td>Du et al., 2005; Fritsche et al., 2007; Kostyuk et al., 2012; John et al., 2013</td>
<td>Shin et al., 2013</td>
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<td>Cell growth and expression of growth factor genes</td>
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<td>Circadian rhythmicity</td>
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<td>Expression of AhR target genes</td>
<td>Wei et al., 2000; Oberg et al., 2005; Scullo et al., 2008; Nair et al., 2009;</td>
<td>Jönsson et al., 2009;  Laub et al., 2010; Wincent et al., 2012; Odio et al., 2013</td>
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<td>2012; Rico-Leo et al., 2013; Sumida et al., 2013</td>
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<td>Genome rearrangement</td>
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<td>Quintana et al., 2008; Veldhoen et al., 2008; Martin et al., 2009; Monteleone et</td>
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<td>Immune response</td>
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<td>Duarte et al., 2013; Wheeler et al., 2013; Zhou et al., 2013</td>
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<td>Nuclear receptor cross-talk</td>
<td>Ekins et al., 2008; Reschly et al., 2008; Bunaciu and Yen, 2013</td>
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Fig. 5. Hypothetical schemes of FICZ homeostasis changes by unbalanced AhR and cytochrome P450 activity. (A) The high-affinity AhR ligand FICZ is constantly produced in the organism. It induces CYP1A activity, which in turn degrades FICZ, thereby creating a homeostatic level of FICZ. (B) Other, high-affinity AhR ligands, in particular the nondegradable TCDD, or very high concentrations of low-affinity AhR ligands can compete for AhR binding, resulting in constantly high CYP1A activity and depletion of FICZ. (C) AhR activation can also occur by factors that block/inhibit CYP1A, leading to exceptionally high FICZ levels because FICZ is no longer degraded. In both scenarios (B) and (C), FICZ homeostatic levels are changed, and aberrant transcription of many genes could occur. The events would be similar for other endogenous high-affinity homeostatic ligands, such as ICZ in the gut.
same studies, an amelioration of experimental allergic encephalitis and experimental allergic arthritis was reported for AhR-deficient mice or for AhR<sup>Δ/Δ</sup> mice (which express a low-affinity AhR allele), adding further to the puzzle. Originally, the phenomena were explained by an induction of Tregs, but reports on direct FoxP3 induction by TCDD in vivo and in vitro are conflicting (Quintana et al., 2008; Duarte et al., 2013), and no enhancement of Tregs was evident in mice engineered to have a constitutively active AhR in T cells (Quintana et al., 2008; Funatake et al., 2009). DCs provide the milieu for T-cell differentiation in vivo, and AhR activation is involved in their tolerogenicity (Hauben et al., 2008; Jin et al., 2012). Conceivably, the situation in vivo integrates AhR activation in a more complex fashion than deduced from in vitro data, and in vitro data must be viewed with caution (Duarte et al., 2013).

Although more data are needed, it is evident that different AhR ligands were beneficial in the experimental model autoimmune diseases. In Table 3, we have listed diseases for which AhR agonists/antagonist have demonstrated promising results in cell or animal studies and which have been suggested as having potential for clinical applications. Many substances and procedures are already protected by patents; however, only few clinical trials exist thus far. Of note in this context, there are drugs that had been approved for human use (thus need no toxicity evaluation in clinical trials) long before their appreciation as AhR agonists or antagonists. Omeprazole and tranilast are examples that are currently under investigation for new applications in the context of their AhR activities (Jin et al., 2012). In addition, although novel AhR-interacting drugs are not yet approved, AhR-activating nutraceuticals, such as I3C, resveratrol, and 3,3′-diindolylmethane, are being marketed.

VII. Conclusions

Organisms must sense and respond to changes in the environment in a meaningful way. The AhR is a protein capable of sensing the (bio)chemical and physical environment. Together with its few high-affinity physiologic ligands, such as FICZ and ICZ, it serves functions in cell proliferation, differentiation and cell functions. The signaling system can be influenced by low-affinity chemicals and even physical stressors such as oxidative stress, which interfere with turnover of the “true” endogenous ligands. In addition, xenobiotic high-affinity ligands can disrupt the system, often to an extent that toxicity ensues, by mimicking or competing with endogenous high-affinity ligands. Barrier organs come in contact with many exogenous chemicals, including human-made pollutants, and chemicals characteristic for bacteria and fungi. Barrier organs are characterized by high cell turnover and express the AhR at high amounts in both their structural and immune cells; indeed, barrier organs have important immune functions. The AhR was demonstrated to have many functions in immune cells and other cells; notably, AhR functions are very cell specific and diverse. The high sensitivity and specificity of AhR signaling are not fully understood. However, available data, in animal models, animal and human cell lines and organotypic cultures, as well as human epidemiology and human studies, suggest high potential for both preventive and therapeutic intervention. More research is needed to understand the full complexity of AhR biology to avoid risks and master the opportunities of therapy and prevention.
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