

Title: More than a cell biosensor. Aryl hydrocarbon receptor at the intersection of physiology and inflammation.

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LIST OF ABBREVIATIONS

AhR	- Aryl hydrocarbon Receptor
ARNT/HIF-1 β	- AhR Nuclear Translocator/Hypoxia-Induced Factor 1 β
AIP	- AhR-Interacting Protein
β -LIP	- Liver-enriched transcriptional Inhibitory Protein isoform β
cAMP	- Cyclic Adenosine MonoPhosphate
CD	- Cluster of Differentiation (cells)
CYP	- Cytochrome P
DRE	- Dioxin Responsive Elements
ECC-1	- Endocervical Cancer Cells type 1
FICZ	- 6-FormylIndole [3,2-b] CarbaZole
HIF	- Hypoxia Inducible Factor
HSP90	- Heat Shock Protein 90
IDO1	- Indoleamine 2,3-DiOxygenase 1
I κ B	- Inhibitory κ B protein
IL-6	- Interleukin 6
IL-6R	- Interleukin 6 Receptor
LPS	- LipoPolySaccaride
MCF	- Michigan Cancer Foundation
NF- κ B	- Nuclear Factor of Kappa light chain gene enhancer in B cell
NC-XREs	- NonConsensus Xenobiotic-Responsive Elements
OSM	- Oncostatin M
PAHs	- Polycyclic Aromatic Hydrocarbons
PG	- Prostaglandin
PMA	- Phorbol 12-Myristate 13-Acetate
pp60src	- PhosphoProtein of 60,000-Da molecular weight from the sarcoma (src) virus
Rel	- Related proteins (including NF- κ B members)
STAT3	- Signal Transducer and Activator of Transcription 3
TCDD	- 2,3,7,8-tetrachlorodibenzo-p-dioxin
TGF- β	- Transforming Growth Factor β
TH17	- T helper 17
Treg	- Regulatory lymphocytes type T
XREs	- Xenobiotic-Responsive Elements

ABSTRACT

Aryl hydrocarbon receptor (AhR), a highly conserved intracellular transcription factor, is activated by a plethora of ligands of both exogenous and endogenous nature. Besides activating xenobiotic metabolizing enzymes, it is involved in the differentiation and development of hematopoietic, hepatic, nervous and immune systems. More and more data describe its role in the regulation of immune responses and in the onset and progression of inflammation. Particularly, established results view AhR as a downstream target of inflammatory molecules, since its transcription is regulated by the inflammatory cascade. Interleukin 6 (IL-6) has been described to sustain early stages of inflammation and to influence the expression of AhR either directly, following STAT3 activation, or in combination with other inflammatory mediators, e.g. Transforming Growth Factor β (TGF- β). In selected inflammatory milieus, once activated, AhR interacts with its targets including the IL-6 promoter, thus originating an auto inflammatory loop.

This perspective review brings together evidence that, in some IL-6-driven pathways, AhR is a downstream target that amplifies the duration and extent of inflammation. Considering that many inflammatory mediators can also trigger the activities of AhR as biosensor and activator of xenobiotics metabolism, this issue is of pivotal importance. The individual susceptibility to some environmental ligands of AhR can be probably explained by considering the individual inflammatory state which could additionally fuel the pro-inflammatory activity of AhR. Thus, AhR could be considered a transducer of a dynamic, bidirectional connection between internal and external environmental stimuli and the inflammatory response.

The helix-loop-helix transcription factor Aryl Hydrocarbon Receptor (AhR) is an evolutionary conserved, ubiquitously expressed biosensor. AhR has a lot of ligands. In addition to natural compounds, of both plant (e.g.: Indoles and Stilbenes) and animal origin (e.g.: Prostaglandin G2 and the tryptophan metabolite 6-FormylIndole [3,2-b] Carbazole (FICZ)), AhR has evolved the ability to bind several compounds of anthropogenic origin, whose amount released into the environment increased in recent decades. These exogenous compounds comprise many polycyclic aromatic hydrocarbons (PAHs), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is the prototypical agonist of AhR.

AhR links the binding of both endogenous and exogenous factors to the regulation of the expression of several genes, including some Phase-I cytochrome P450 enzymes such as Cyp1a1, Cyp1a2, Cyp1b1 and Phase-II enzymes such as Glutathione-S-transferase (14). AhR interacts also with a relevant number of cell pathways and gene sets (20), including those expressing some proteins that regulate the main phases of the cell lifecycle, such as growth, differentiation, proliferation and apoptosis (1). In the absence of ligands, two proteins of the Heat Shock Proteins 90 (HSP90) family, the AhR-interacting protein (AIP), the co-chaperone protein p23, and the protein tyrosine kinase pp60src associate in the cytoplasm with AhR and maintain it in a "resting state". Following ligand binding, this chaperone complex translocates to the nucleus. Here, the inactivating complex splits and AhR binds to AhR Nuclear Translocator/Hypoxia-Induced Factor 1 β (ARNT/HIF-1 β). Finally, the new complex AhR/ARNT interacts with the xenobiotic-responsive elements (XREs) of target genes (16).

After releasing ARNT, AhR exits nucleus and is degraded by the cytoplasmic 26S proteasome system (4), leading to the termination of the AhR response (Figure 1). In a physiological context, AhR activation by cAMP was described in 2005 (13). Alternative, "non-canonical" signaling has been recently described (5, 24). In particular, it has been observed that the promoter of some AhR-responsive genes contains nonconsensus XREs (NC-XREs) (23). Moreover, similarly to other transcription factors, it has been demonstrated that AhR interacts with mobile genetic elements (12).

Accumulating evidence has established that AhR participates in immune system regulation and inflammation. In 2008, the Quintana group pointed out that AhR can influence TH₁₇

and T_{reg} cell differentiation with ligand-specific modalities (15). In fact, different AhR ligands can behave differently in the regulation of the immune system. For instance, TCDD activates T_{reg} cells, thus quenching autoimmune processes. On the contrary, FICZ, a potent endogenous activator of AhR, favors autoimmune processes. A growing body of literature has pointed out the involvement of AhR upstream of inflammatory processes (6, 2). Specifically, AhR regulates the transcription and the expression of inflammatory molecules. In this context, either activated by ligand binding or not, AhR interacts with endogenous molecules, including NF-κB. For example, in mouse hepatoma cells, ligand-activated AhR and RelA (the p65 subunit of NF-κB) physically associate. In this way, NF-κB trans-represses AhR activity and, in the meantime, AhR hinders the binding between I-κB sequences and NF-κB, thus affecting its functioning in the resolution of inflammation (18, 19) (Figure 2). As a matter of fact, it is known that the p65-AhR•ligand complex does not interact directly with the XRE, while this happens when AhR•ligand is associated with nuclear ARNT. Intriguingly, in the cytosol and nucleus of lung cancer cells, RelA associates with unstimulated and overexpressed AhR. Once in the nucleus, the complex RelA-AhR binds to the κB element in the IL-6 promoter. Consequently, NF-κB reporter activity, IL-6 expression and the inflammatory process are upregulated (3). NF-κB activation can be also obtained in a classical scenario. Here, TCDD evokes an AhR-ARNT/XRE-mediated nuclear activity that usually includes NF-κB among the downstream mediators (18). In a different experimental context, the Vogel group described the possible role of AHR/Relβ interaction in the resolution of inflammation (22). A frequent inflammatory partner of AhR is IL-6. In LPS-inflamed bone marrow cells, a model of innate immunity, AhR inhibits IL-6 expression by preventing NF-κB binding to the IL-6 promoter sequences (9). On the other hand, in MCF-7 breast and ECC-1 endocervical cancer cells, the co-administration of TCDD and IL-1β or TCDD and phorbol 12-myristate 13-acetate (PMA) stimulates the binding of AhR to the IL-6 promoter (8).

In contrast, there are few data reporting that AhR is transcriptionally regulated by IL6. In 2008, Veldhoen studied the involvement of AhR in human and murine CD4⁺T cells under TH₁₇ polarizing conditions. Here, he observed the induction of AhR following the co-administration of IL-6 and TGFβ. This is the first report describing the induction and the activation of AhR downstream of inflammatory conditions (21).

By expanding the role of AhR, this is an innovative perspective. Following Veldhoen's report, this view was not further substantiated until in 2013 the Stobbe-Maicherski group added new data supporting the hypothesis of AhR as a downstream effector of IL-6. They reported that in HepG2 cells the administration of oncostatin M (OSM), a multifunctional cytokine belonging to the IL-6 family group, increases AhR transcription and protein expression, thus making inflamed cells more prone to respond to xenobiotics and environmental pollutants. They proved that STAT3 proteins, which are the main effectors of the IL-6 cytokine family, bind to a STAT motif in the AHR promoter, thus enhancing AHR gene transcription and protein synthesis. This induction affects the expression of both inducible and constitutive AhR and is dose and time dependent (17). In summary, these data suggest that OSM, and possibly the whole IL-6 cytokine family, modulate AhR expression through activated STAT3, independently of NF-κB involvement. They also confirmed previous reports (7, 11) showing that OSM mediates the repression of CYP1A1 transcription.

To corroborate this new role of AhR, in 2014 Litzenburger and co-workers suggested that in cancer cells kynurenine, an endogenous agonist of AhR, is the link between IL-6 and the AhR pathway through STAT3 activation. They showed that in an IL-6-driven inflammatory environment, following IL-6R activation, STAT3 is phosphorylated and stimulates the expression of Indoleamine-2,3-dioxygenase (IDO1). This enzyme catalyzes the synthesis of new molecules of kynurenine. Kynurenine, in turn, binds and activates AhR, which interacts with the promoter of the IL-6 gene in the nucleus. IL-6 is thus produced, secreted and subsequently binds to IL-6R. Upon IL-6R activation, STAT3 is phosphorylated, acetylated and interacts with the promoter of target genes, among which IDO1, thus completing an autocrine IDO-AhR-IL-6-STAT3 auto-

inflammatory loop (10) (Figure 3). The activation of this pathway affects T cell proliferation and induces an immunosuppressive effect. Here, AhR plays a pivotal role, as it links the endogenous kynurenine signaling to IL-6 synthesis. IL-6, in turn, instigates the activation of a downstream pathway, which sustains kynurenine production.

Both studies have been carried out in tumor cell lines, a model that is widely used to study cell biology, but severely limits the translation of data into a physiological context. Therefore, our group is currently evaluating the actual weight of IL-6-induced acute inflammation on AhR activation in a model of non-tumorigenic human breast cells. We hypothesize that AhR is a downstream effector of IL-6-induced inflammation and that, once activated, it induces the transcriptional activation of IL-6. Thus, the recruiting of AhR can extend and amplify the inflammatory state. In relation to this scenario, we propose that when non-tumorigenic breast cells are inflamed with IL-6, AhR can behave as a hub which sustains an auto-inflammatory, self-sustained loop.

PERSPECTIVES

In this synthetic overview, we bring together evidence that AhR functions are not strictly confined to the environmental toxicology area, where its first classification as xenobiotic biosensor would place it. Being able to bind to both endogenous and exogenous molecules, it participates to several physiologic functions, including inflammation. In this context, several research groups have tethered its activation to the expression of inflammatory markers, such as IL-6 (8). Besides these inflammogenic properties, we have reported recent data highlighting the transcriptional regulation of AhR by inflammatory agents, in particular IL-6 cytokine family (17). It is known that chronic inflammation is the key component in a large array of pathologies (2). Keeping this in mind, we propose that AhR is not simply a biosensor for xenobiotics, but also a feasible downstream target of inflammatory molecules. The new perspective we advance is that AHR activation is not only able to induce inflammation, but is by itself induced by few inflammatory mediators, such as IL-6. Finally, we suggest that the mutual interplay between IL-6 and AhR can generate an auto-inflammatory and self-sustaining loop, with a significant role both in the onset and in the maintenance of inflammatory chronic states. Considering that AhR effects are cell and ligand-specific and, as described, are significantly shaped from the inflammatory microenvironment, we believe that this perspective is, at the same time, challenging and extremely promising.

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

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LEGENDS

Fig. 1. Canonical signaling pathway of AhR.

The inactive form of AhR is found in the cytoplasm, in a complex with two Heat Shock Proteins 90 (HSP90), the AhR-interacting protein (AIP), the cochaperone protein p23, and the protein tyrosine kinase pp60src. After ligand binding, it moves to the nucleus, where it releases these inactivating molecules. Here, it associates with AhR nuclear translocator (ARNT), forming a heterodimer which interacts with the Xenobiotic Responsive Elements (XREs) in the promoter region of target genes expressing Phase I/Phase II enzymes. Afterwards, AhR/ARNT complex separates and AhR comes back to the cytoplasm, where it is degraded from the ubiquitin-mediated 26S proteasome pathway.

Fig. 2. Reciprocal antagonistic modulation of AhR and NF- κ B.

In mouse hepatoma cells treated with Ahr ligands and inflammatory ligands of NF- κ B, AhR and RelA (the p65 subunit of NF κ B) physically associates. NF- κ B trans-represses AhR activity, as evidenced by the suppression of CYP1A1 and CYP1A2 activity, while AhR hinders the binding between I- κ B sequences and NF- κ B.

Fig. 3. Schematic view of the IL-6-STAT3-(AhR)-IDO-AhR-IL6 auto-inflammatory loop.

In IL-6- driven inflammatory environment, IL-6 receptor (IL6R) is activated. After Janus Kinase (JAK)- mediated phosphorylation, STAT3 is activated, moves into the nucleus and interacts 1) with a putative STAT motif in AHR promoter region, thus sustaining AHR gene activation and expression 2) the promoter of the enzyme indoleamine-2,3-dioxygenase (IDO). Cytoplasmic IDO degrades tryptophan (Trp) and produces kynurenine (Kyn), which is an endogenous ligand of AhR. Activated AhR translocates into the nucleus and binds to the XREs in the promoter of IL-6 gene. Finally, IL-6 is produced and released from the cell. IL-6 binding to IL-6R completes this auto inflammatory, AhR-sustained loop.





