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Ligand activation of the Ah receptor contributes to gastrointestinal homeostasis

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Abstract

The Ah receptor (AHR) is capable of binding a structurally diverse group of compounds that can be found in the diet, produced by bacteria in the gut and through endogenous metabolism. The gastrointestinal tract is a rich source of AHR ligands, which have been shown to protect the gut upon challenge with either pathogenic bacteria or toxic chemicals. The human AHR can be activated by a broader range of ligands compared to the mouse AHR, suggesting that studies in mice may underestimate the impact of AHR ligands in the human gut. The protective effect of AHR activation appears to be due to modulating the immune system within the gut. While several mechanisms have been established, due to the increasingly pleiotropic nature of the AHR, other mechanisms of action likely exist that remain to be identified. The major contributors to AHR function in the gut and the most appropriate level of receptor activation that maintains intestinal homeostasis warrants further investigation.

Graphical abstract

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Nothing declared

Keywords

Ah receptor; AHR; indole; IL22; intestine; gastrointestinal

Introduction

Evidence suggests the predominant biological activities of the AHR are evoked through ligand binding. Thus, one key aspect of AHR research has centered upon the identification of the endogenous ligand for this receptor. However, the characterization of multiple, structurally diverse physiological AHR ligands have led to a complex story likely to be context specific. It is now apparent that a biological ‘cocktail’ of endogenous, pseudo-endogenous¹ and dietary AHR ligands exist and that spatiotemporal availability, generation (or metabolic elimination) and competition between disparate ligands represent the major factors dictating the physiological activities and functions of the AHR. Importantly, emerging evidence highlights the physiological relevance of intrinsic AHR ligand activation beyond xenobiotic metabolism.

Dietary AHR ligands

In essence, an organisms’ diet comprises a complex mixture of xenobiotics, some of which provide nutritional and/or other physiological value while others may be toxins. Barring environmental, occupational or lifestyle (smoking) exposure to dioxins *etc.*, the diet is now recognized as a major source of AHR ligands. The AHR has a promiscuous capacity to bind and respond to raw and cooked dietary components; including those derived from plants *e.g.* fruits and cruciferous vegetables (Figure 1) [1–7]. AHR ligation by dietary components likely serves to prime phase I cytochrome P450 metabolism within the gastrointestinal tract and through hepatic first-pass metabolism at the onset of ingestion to detoxify/eliminate potentially harmful dietary constituents [8]. In addition, the emergence of AHR as a pleiotropic factor with activities beyond metabolism suggests that dietary AHR ligands likely influence a wide range of physiological processes such as immune surveillance and microbiota/host interactions [9–11].

The most widely investigated dietary AHR ligands are those generated from the plant glucosinolates such as glucobrassicin a component of cruciferous vegetables. Mechanical breakdown facilitates myrosinase-dependent hydrolysis of glucobrassicin to a number of products, including indole-3-carbinol (I3C) and indole-3-acetonitrile (I3ACN), both of which exhibit AHR agonist activity [7,12–15]. Within the acidic environment of the stomach, I3C is susceptible to acid condensation yielding indolo[3, 2*b*]carbazole (ICZ), and 3, 3’-diindolymethane (DIM), both of which exhibit AHR binding potential [13,14,16,17]. These acid condensation products may represent the dominant AHR ligand binding activity *in vivo* when compared to I3C [18]. Pharmacokinetic data points to rapid absorption but low systemic retention of I3C, whereas ICZ, and DIM are present at steady-state levels due to a combination of higher chemical stability and longer plasma half-life relative to I3C [19,20].

¹Pseudo-endogenous AHR ligands are defined as any metabolite that is produced by the microbiota and are capable of binding to the AHR.

In addition, numerous other dietary constituents including flavones, isoflavones, flavanones, and carotenoids have been shown to elicit AHR activity and are either known or inferred AHR ligands [21–24]. It is clear that the diet represents a rich source of AHR ligands, however this generates a degree of complexity, which makes it difficult to determine the relative contribution of individual AHR ligands with regard to overall physiology. Although ICZ exhibits high-affinity (low nM) AHR binding similar to that exhibited by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the majority of the dietary AHR ligands identified to date are weak ligands by comparison. Additional complexity due to the fact that, despite being AHR ligands, many are characterized as AHR antagonists with regard to canonical AHR signaling. In addition, those that exhibit weak agonist activity may, if present at sufficient concentrations, act as competitive antagonists of more potent ligands *e.g.* flavonoids [25]. Although manifesting as AHR ligands, it is likely that many dietary components influence signaling through AHR-independent means, thus their contribution to physiology through AHR binding is difficult to interpret, particularly *in vivo*.

Tryptophan, a precursor to AHR ligands

A theme has emerged, which highlights a central role for the essential amino acid tryptophan as a major physiological reservoir for the synthesis of AHR ligands. A large body of evidence has demonstrated that tryptophan can undergo a range of spontaneous and enzyme-catalyzed conversions provided by the host and its associated microbiota to yield numerous physiologically relevant AHR ligands. Early observations indicating enhanced *in vitro* and *in vivo* AHR activity following exposure to ultra-violet radiation implicated light as a factor associated with endogenous AHR ligand production [26–28]. A series of studies identified a number of UV-dependent tryptophan photo-oxidation products, including 6-formyl and 6, 12-diformylindolo[3, 2*b*]carbazole (FICZ and dFICZ, respectively) as specific, high-affinity AHR ligands (Figure 1) [27,29–32]. Recently, evidence demonstrating UV-independent conversion of tryptophan or its metabolites to FICZ under conditions of oxidative stress implicates a route for extra-dermal AHR ligand generation in the absence of light [33]. In contrast to the spontaneous and therefore unregulated generation of UV-mediated tryptophan photo-oxidation products, studies have identified the major physiological route of tryptophan metabolism as a source of AHR ligands. Processing through the kynurenine pathway accounts for > 90% of tryptophan metabolism and is initially catalyzed by isoforms of the functionally homologous tryptophan-2, 3-dioxygenase (TDO1/2) and indolamine-2, 3-dioxygenase (IDO1/2) enzymes [34,35]. Induction of TDO or IDO within the liver or periphery respectively, leads to tryptophan localized depletion and the production of kynurenine, kynurenine-, xanthurenic- and cinnabaric acids, each of which displays AHR ligand mediated activity (Figure 1 and 2) [36–39]. Studies have revealed the physiological link between tryptophan and the AHR to be more complex than previously envisioned, with a bidirectional axis existing in which tryptophan metabolites modulate AHR activity and the AHR modulates tryptophan metabolism. The expression of IDO, particularly during inflammatory stress through Toll-like or IFN γ receptor ligation, is elevated through the combinatorial action of STAT1, NF κ B and the AHR, thus generating a kynurenine pathway-AHR-IDO-AHR autocrine loop [40–42].

Pseudo-endogenous AHR ligands

Barrier tissues provide a niche for complex microbial ecosystems. These site-specific microbial populations cooperate and compete with the host organism to generate a dynamic metabolic landscape rich in metabolites, the physiological relevance of which is only now being appreciated and investigated. Evidence supports microbial tryptophan metabolism, particularly within the gastrointestinal tract, as a site for *in vivo* AHR ligand generation (Figure 1 and 3) [15,43,44]. Bacterial tryptophan uptake, facilitated by transporters, initiates catabolism through tryptophanase and downstream enzymes to generate a succession of tryptophan metabolites that may ultimately modulate AHR function *in vivo*. Many of the microbiota-derived tryptophan metabolites, including indole, indole-3-acetic acid, indole-3-aldehyde have been demonstrated to be AHR ligands [11,45,46]. Given the abundance and extensive metabolic capacity of bacteria, particularly within the gastrointestinal tract, such ligands are likely present at sufficient concentrations to stimulate AHR activity [43]. Additional tryptophan metabolites including malassezin, tryptanthrin, tryptamine, and 3-methyl indoles are apparent AHR ligands and influence AHR activity, typically assessed through induction of AHR transcriptional targets [43–45,47–50]. Downstream metabolism of microbiota-derived tryptophan metabolites can generate additional AHR ligands *e.g.* indole can undergo hepatic CYP2E1-mediated conversion to indoxyl, a precursor to indoxyl sulfate, indirubin and indigo, each of which exhibits AHR ligand activity [51–53]. Due to its capacity to bind and respond to bacteria-derived tryptophan metabolites, it is suggested that the AHR may contribute to the surveillance of the microbiota. Although not examined in the context of microbial AHR ligands, studies identify a reduced microbial burden within the gastrointestinal tract of mice exposed to dietary AHR ligands [54]. While, the microbiota is now established as a determinant of overall physiology and associated with many disease states, the contribution of AHR ligand generation by the microbiota is only now being investigated.

Inter-species variability and intrinsic AHR ligands

Despite the evolutionary conservation of AHR expression from invertebrates to mammals, the gene encoding AHR has undergone functionally significant divergence across and within species [55–57]. Evidence highlights a marked variability regarding the potential for a given ligand to induce AHR activity across different species *e.g.* indirubin, indoxyl sulfate, indole, kynurenine, kynurenic acid and xanthurenic acid all exhibit greater potential to activate the human AHR when compared to mouse AHR [37,43,53,58,59]. Factors independent of the AHR likely contribute to the variation in AHR ligand-mediated physiology across species. However, evolutionary divergence of functionally critical residues within the AHR protein, particularly the ligand binding domain, likely represent the dominant determinant. *In vivo* studies performed in rodents supporting a role for intrinsic AHR ligands in physiology, particularly immune regulation, may not present an accurate representation of AHR ligand function in humans. Indeed, data demonstrating that many endogenous and pseudo-endogenous AHR ligands have greater affinity for the human AHR than that of mouse would argue that human sensitivity and physiological responses to such ligands are underestimated. The enhanced sensitivity of the human AHR for such endogenous and pseudo-endogenous ligands relative to rodent AHR is in stark contrast to the situation with exogenous xenobiotic

toxicants such as TCDD, which exhibit greater than ten-fold increased affinity for rodent AHR species [60]. Thus, in contrast to the potential underestimation of endogenous ligand-mediated AHR activity, the toxic effects of TCDD and other xenobiotic pollutants to humans are likely overestimated.

AHR and immune signaling

Systemically, activation of the AHR has been shown to either enhance or repress inflammatory signaling and inflammation in a context and cell type specific manner [61–63]. However, in the gastrointestinal tract most studies support an anti-inflammatory role for ligand mediated AHR activation. While the level of AHR activation and the type of AHR ligands present also will likely play a role in the response observed. It is important to point out that many of the studies that propose that the AHR is anti-inflammatory utilized *Ahr*^{−/−} mice and thus were independent of ligand activation status or the ability of the AHR to exhibit activity in the absence of ligands [64]. Part of the maintenance of a normal host-microbiota axis in the gut involves proper immune surveillance by the host. For example, cytokines and chemokines are pleiotropic factors that are key mediators of innate immunity and barrier function. Indeed, the AHR has been shown capable of modulating expression of *Il1b*, *Il6*, *Ccl20*, *Ccl1* [65–68]. In addition, prostaglandin G/H synthase 2 (*Ptgs2*) is also directly regulated by the AHR in combination with other transcription factors [69]. Considering the importance of prostaglandin E2 produced by PTGS2 to maintaining gut homeostasis, the role of the AHR in this pathway needs to be further explored [70]. Thus, the production of AHR ligands either by microbiota or by immune cells could act as a sensor mechanism leading to heightened immune surveillance.

Upon enhanced expression of inflammatory cytokines in the intestinal tract, retinoic acid, along with activation of the AHR, can mediate a dramatic increase in Th17 cell development from CD4⁺ T cells. Th17 cells express both IL17 and IL22 while AHR agonist treatment leads to an increase in IL22 expressing cells [71,72]. IL22 is an important cytokine in wound healing through stimulating survival of intestinal stem cells and enhancing anti-bacterial peptide production from epithelial cells [73,74]. The precise mechanism of AHR-mediated IL22 expression is not clear, however AHR agonist can stimulate IL22 expression in cultured CD4⁺ T cells in a Notch-dependent manner [75]. Another proposed mechanisms of IL22 induction is through AHR-induced IL23 expression in infected macrophages, which in turn stimulates T cells to produce IL22 [65]. Importantly, the endogenous AHR ligand cinnabarinic acid has been shown to induce IL22 production in CD4⁺ T cells placed in conditions that lead to Th17 T cell polarization [38].

Intestinal challenge models and AHR ligands

Dextran sodium sulfate (DSS) exposure is often used as a mouse model to study factors that influence inflammatory bowel disease and there is some evidence that this model is relevant to humans [76]. Potent AHR ligands (e.g. TCDD, FICZ, β -naphthoflavone) have been shown to attenuate the severity of symptoms from DSS exposure [77–81]. In these studies a number of possible mechanisms for AHR mediated repression of DSS-mediated toxicity were suggested, including an increase in IL22, PGE₂, and regulatory T (Treg) cell levels.

Dietary administration of I3C or DIM lessens DSS-mediated colonic inflammation and disease severity, suggesting that production of AHR ligands such as ICZ are protective through AHR activation. This would suggest that foods rich in AHR ligands or ligand precursors would protect against chemically-induced gastrointestinal injury. Dietary cruciferous vegetables are capable of inducing AHR activity and have been shown to attenuate DSS induced toxicity, however, whether these effects are mediated by AHR activation has not been established [82]. The probiotic bacteria *Propionibacterium freudenreichii* produces the AHR ligand 1,4-dihydroxy-2-naphthoic acid, which has been shown to inhibit DSS-induced colitis [83]. This illustrates the possibility that intestinal bacteria may be capable of producing AHR ligands to improve intestinal health.

Ahr^{-/-} mice are more susceptible to *Listeria monocytogenes* and *Citrobacter rodentium* infection compared to *Ahr*^{+/+} mice [84,85]. These studies, along with the general chemopreventive properties of AHR ligand treatment on gastrointestinal health would suggest that AHR ligands in the diet may protect against opportunistic bacterial infections. Indeed, a recent study demonstrated that dietary I3C can decrease the level of *Clostridium difficile* induced lethality in mice, in part through activation of the AHR [86]. It will be important to test other AHR ligands in this infection model. Macrophages are a critical cell type in the intestinal tract directly involved in clearance of bacteria. AHR expression is critical to maintain macrophage survival in the presence of pathogenic bacteria and for robust production of reactive oxygen species (ROS) [87]. In addition, treatment with induces ROS in mitochondria [88]. Studies in *Mycobacterium tuberculosis* infected macrophages have revealed that activation of the AHR by the endogenous ligand kynurenine reduced bacterial viability [65]. Treatment with kynurenine also induced IL23A in macrophages, which in turn can lead to IL22 production by intraepithelial lymphocytes. These observations would support the concept that the presence of dietary- or bacterially-generated AHR ligands may also aid in the response to pathogenic bacteria.

The 2,4,6-trinitrobenzene sulfonic acid (TNBS)- induced colitis model is widely used to mimic Crohn's disease and CD4+ T cells play a critical role in the development of disease in this model. Treatment of mice orally with TCDD in the TNBS murine model resulted in decreased inflammation and an increase in Treg cells, which may in part explain the mechanism mediating inhibition of inflammation [89]. FICZ has also been shown to significantly reduce inflammation in a TNBS model, while treatment with an AHR antagonist increases colitis severity [79]. A possible mechanism of these observations is the ability of AHR ligand to increase IL22 expression. A likely mechanism of Treg production is the ability of AHR ligand to induce IDO1 and IDO2 expression in dendritic cells [41].

Conclusions

Studies in rodents clearly support the concept that ligand activation of the AHR is protective from toxic insults that mimic chronic and acute gastrointestinal diseases in rodent models. From a mechanistic standpoint these results appear to be due at least in part to an increase in intraepithelial lymphocyte residence time, elevated IL22 expression and expansion of Treg cell levels within the intestinal tract. While treatment with relatively high levels of AHR levels is chemoprotective, it is not clear whether this protection is conferred from dietary

consumption of AHR ligands found in foods consumed in a healthy diet. The role of AHR antagonist, selective AHR modulators, and agonist activities in the gut will need to be further explored. Can the microbiota produce enough AHR ligands to be protective in the intestinal tract, what are the precursors that can lead to AHR ligand production? These questions will need to be addressed in order to assess how knowledge of AHR ligands can lead to selecting foods or therapeutic agents to maintain intestinal and overall health. One important point to consider is that dietary ligands may exhibit the greatest activity in the small intestine prior to absorption, while microbiota generated ligands would be predominantly in the cecum and large intestine. Another issue that has not been adequately addressed is the role of the AHR in intestinal cells of epithelial origin. To further complicate studies on endogenous and microbiota generated ligands is the difference in ligand specificity between the human versus mouse AHR, suggesting that the human AHR may be more responsive to many of these ligands[90]. This observation would indicate that an appropriate humanized AHR mouse model is needed to address these issues. Yet another issue that needs to be considered further is whether high AHR ligand exposure can shift T cell populations to an immune tolerant status, which would be advantageous to attenuate autoimmune diseases. However, this attenuating effect in turn might increase susceptibility to cancer (e.g. colon cancer), and thus this issue should be considered in future studies [91].

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Figure 1.
Sources and examples of known dietary, endogenous and microbiota-derived AHR ligands.

Figure 2. The kynurenine pathway as a source of endogenous AHR ligands

Host tryptophan metabolism through the activity of hepatic tryptophan 2, 3-dioxygenase (TDO) or peripheral indolamine 2, 3-dioxygenase (IDO) yields kynurenine, an AHR ligand. Subsequent metabolism of kynurenine by kynurenine monooxygenase (KMO), kynureninase (KYNU) and kynurenine aminotransferase (KAT) provides additional AHR ligands. These AHR ligands have the capacity to further enhance expression of IDO in an autocrine loop. Activation of the kynurenine pathway is associated with increased immune tolerance and may contribute to gastrointestinal homeostasis.

Figure 3. Tryptophan metabolism by the gastrointestinal microbiota represents a source of AHR ligands and ligand precursors

The uptake of luminal tryptophan by the resident gastrointestinal microbiota through amino acid transporters e.g. Mtr, TnaB and AroP provides the substrate for enzymatic conversion by bacterial monooxygenases (IaaM), acetamide hydrolases (IaaH), decarboxylases to generate AHR ligands. In addition, microbial tryptophanase (TnaA) activity can generate the AHR ligand indole. Efflux of bacterial indole by transporters e.g. AcrE and AcrF provides a substrate for host metabolism e.g. CYP2E1 and sulfotransferases (SULT) resulting in the generation of additional AHR ligands and ligand precursors. The repertoire of tryptophan-derived microbial AHR ligands has the capacity to influence host-microbe homeostasis through the AHR-dependent modulation of IL22 expression and immunosuppressive Treg differentiation.