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Use of natural AhR ligands as potential therapeutic modalities against inflammatory disorders

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Abstract

The aim of this review is to discuss research involving ligands for the aryl hydrocarbon receptor (AhR) and their role in immunomodulation. While activation of the AhR is well known for its ability to regulate the biochemical and toxic effects of environmental chemicals, more recently an exciting discovery has been made indicating that AhR ligation can also regulate T-cell differentiation, specifically through activation of Foxp3⁺ regulatory T cells (Tregs) and downregulation of the proinflammatory Th17 cells. Such findings have opened new avenues of research on the possibility of targeting the AhR to treat inflammatory and autoimmune diseases. Specifically, this review will discuss the current research involving natural and dietary AhR ligands. In addition, evidence indicating the potential use of these ligands in regulating inflammation in various diseases will be highlighted. The importance of the AhR in immunological processes can be illustrated by expression of this receptor on a majority of immune cell types. In addition, AhR signaling pathways have been reported to influence a number of genes responsible for mediating inflammation and other immune responses. As interest in the AhR and its ligands increases, it seems prudent to consolidate current research on the contributions of these ligands to immune regulation during the course of inflammatory diseases.

Keywords

AhR ligands; aryl hydrocarbon receptor (AhR); inflammation

INTRODUCTION

Evidence supports the notion that the aryl hydrocarbon receptor (AhR) plays an important role within biological systems across several species. Initially, the AhR was thought to be involved predominantly in the metabolism of environmental chemicals. More recently, however, researchers and clinicians have become interested in the role of the AhR in immune functions, particularly in the realm of inflammation. The expression of the AhR in a

majority of immune cell types and the expression of multiple xenobiotic- or dioxin-responsive elements (XREs/ DREs) in the promoter region of many genes that regulate the immune response demonstrates the importance of this receptor in immunological processes.¹ Since the immune system was essentially normal in the AhR-null mice, it may appear that AhR is not essential for the development of a functional immune system.² However, other studies have demonstrated that ligation of AhR during developmental stages may lead to profound immunosuppression.³ Thus, the normal development of the immune system in AhR-null mice may result from compensatory mechanisms. Essentially, the effects exerted by ligand-induced AhR activation on the immune system are determined by a number of varying factors, which include the cell types involved, their activation status, and the type of antigen stimulation.

The aim of this review is to discuss research that addresses AhR ligands and their role in immunomodulation. Specifically, current research on natural and dietary AhR ligands will be discussed. In addition, evidence indicating the potential of these ligands to regulate inflammation in various diseases will be highlighted.

History and discovery of the AhR

The AhR, a member of the family of basic helix-loop-helix transcription factors, was first identified and investigated almost 30 years ago. Phylogenetic analysis revealed the *AhR* gene has been highly conserved throughout evolution.⁴ The *AhR* gene has been found in various cell types present in mammals, amphibians, reptiles, and birds.⁵ The fact that the *AhR* gene has been so highly conserved provides evidence of the fundamental importance of AhR in biological systems. Early studies in the 1970s revealed AhR to be a key regulator in the metabolism of foreign chemicals, commonly referred to as xenobiotics. Researchers discovered a mechanism by which the AhR can direct the metabolism of xenobiotics by increasing the levels of cytochrome P450 enzymes, which then causes biotransformation and increases the water solubility of foreign chemicals, which leads to their expulsion.⁶ On the other hand, some polycyclic aromatic hydrocarbons such as benzo(a)pyrene have been shown to be metabolized by cytochrome P450 into carcinogens and teratogens.⁷ Since the early studies, the role of the AhR in metabolism and toxicity has been well established. However, more recently, scientists have made an exciting discovery that the AhR may also play a vital role in the regulation of the immune response.

AhR structure, function, and signaling

The basic structure of the AhR consists of an N-terminal basic helix loop helix (bHLH) domain, a C-terminal variable domain, and a central PER-ARNT-SIM (PAS) domain with two degenerate repeats, known as PAS-A and PAS-B (Figure 1).^{8–10} The N-terminal bHLH serves as the DNA-binding domain and helps support dimerization.¹¹ The extremely variable, which accounts for differences in receptor molecular weight between species.^{10,12,13} The PAS domain is involved in DNA recognition, ligand binding, and chaperone interactions. This domain, along with the bHLH region of the N-terminus, helps mediate heterodimerization of the AhR with the aryl hydrocarbon receptor nuclear translocator (ARNT).^{14,15} The ligand-binding site of the AhR is structurally flexible and allows for a plethora of diverse ligands.¹⁶

In its unactivated form, the AhR is normally found in the cytosol, chaperoned by heat-shock protein 90 (Hsp90), p23, X-associated protein 2 (XAP2), and AhR-associated protein 9 (ARA9) (Figure 2).¹⁷ Hsp90 helps preserve the AhR in a conformational state that is able to bind ligand and prevent unsolicited translocation of the AhR into the nucleus. The phosphoprotein p23 assists in the interaction between AhR and Hsp90. The ARA9 protein is involved in the proper folding of AhR in the cytoplasm.^{18–20} The ligand-binding pocket of the AhR is capable of interacting with a sundry of ligands with structural and physicochemical heterogeneity.¹⁷ It is important to note that the three-dimensional structure of the AhR ligand-binding domain has not been elucidated, and therefore the mechanisms underlying ligand diversity and receptor conformational changes following binding remain unknown. Thus, in addition to differences in the AhR expressed in various species and tissues, the ligands may also induce a wide range of biological responses. In the event of ligand binding to the AhR, the receptor undergoes structural modifications in which a nuclear localization sequence becomes exposed.⁴ It is generally believed that this receptor-ligand complex then translocates to the nucleus, where ARNT replaces Hsp90 and forms a heterodimer with the ligand-bound AhR.¹⁷ This heterodimer then binds to *cis* elements of DNA that contain XREs/DREs. XREs/DREs possess a consensus sequence (5'-TNGCGTG-3') that can be found in the promoter regions of a large number of target genes, including cytochrome P450 enzymes (CYP1A1, CYP1A2, CYP1B1), glutathione S-transferases, uridine diphosphoglucuronosyltransferases, NAD(P)H-dependent quinone oxydoreductase-1, aldehyde dehydrogenase 3A1, and breast cancer resistance protein genes.^{21–23} Once bound to XREs/DREs, the AhR/ARNT heterodimer acts as a transcriptional complex that can regulate and modify transcriptional activity and chromatin structure.²⁴ The regulation of ligand-bound AhR is strictly controlled in the cell via two main mechanisms. The first occurs after the ligand-bound AhR enters the nucleus and associates with the XRE/DRE region and is then exported from the nucleus and proteolytically degraded within approximately 4 h of ligand-AhR interaction.²⁵ The second mechanism involves the AhR repressor protein, which is structurally analogous to AhR but does not require interaction with ligand prior to binding with ARNT. AhR repressor protein is upregulated upon AhR activation and manages AhR function by acting as a transcriptional repressor.²⁶

Research has shown that the AhR plays a significant role in a variety of different cellular and developmental processes. The AhR can control various functions, including the development of the nervous system and vasculature as well circadian rhythms, metabolism, and stress responses to hypoxia.⁴ The AhR can also cross-talk and directly interact with proteins involved in major signaling pathways such as NF- κ B and various kinases such as src, c-JUN N-terminal kinase (JNK), p38, and mitogen-activated protein kinase (MAPK).²⁷ In addition, the AhR can play a role in targeting proteins for proteasomal degradation by acting as a ligand-dependent E3 ubiquitin ligase.²⁸ Evidence supports that the AhR is capable of interacting with the retinoblastoma protein to help prevent cancer growth²⁹ and the estrogen receptor to mediate estrogen metabolism.^{30,31} In fact, the AhR is known to be expressed in a majority of cells within the immune system, such as lymphocytes (T cells and B cells), macrophages, dendritic cells, granulocytes, and natural killer cells, and its precise role in immune functions is becoming clearer.³²

The AhR and its homologs have been identified in various species of animals, including invertebrates, fish, birds, and mammals. While most animals express only one AhR isoform, fishes and birds have been shown to have two: AhR1 and AhR2. The importance of the AhR in biological systems was highlighted with the development of AhR-deficient mice using homologous recombination techniques that involved the replacement of certain exons of the *AhR* gene with a neomycin resistance gene.³³ Of the current strains of AhR-null mice available, one strain contains a deletion in exon 1 (1/1) and the other strain has a deletion in exon 2 (2/2). In the AhR^{1/1} strain, there is an absence of the translational start site for AhR expression and part of the DNA binding site, which leads to subsequent deletion of AhR mRNA and protein expression.³⁴ The other strain, designated AhR^{2/2}, contains a deletion of the bHLH domain and therefore cannot bind ARNT and the DNA consensus region, resulting in the expression of AhR mRNA, but not AhR protein.^{34–36} All of these AhR-null mice have been shown to exhibit various abnormalities.² Such abnormalities include reduced life expectancy, lower body weight, and decreased liver function due to a defective development in liver vasculature. AhR-deficient mice display fibrotic and inflammatory lesions as well as abnormal hematopoietic development.^{37,38}

AhR ligands

Over the years, a number of AhR ligands, both synthetic and natural, have been discovered. The identification of these ligands has provided increased knowledge of the physiological functions of the AhR. AhR ligands can vary greatly in their chemical structure and binding affinities, ranging from 10^{-12} and 10^{-3} M (Figure 3).^{39–51} Those AhR ligands which possess a greater affinity for the receptor are often deemed more toxic and may gain entry into the lungs by inhalation as well as through the skin.⁷ These AhR ligands, typically components of environmental pollutants, include the halogenated aromatic hydrocarbons such as polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls as well as polycyclic aromatic hydrocarbons such as benzo(a)pyrene, anthracene, and 3-methylcholanthrene. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD), a halogenated aromatic hydrocarbon, is one of the most immunotoxic and immunosuppressive AhR ligands. Due to its high binding affinity, TCDD has become the prototype for studying the bioactivity of the AhR. However, researchers have been searching for additional, less toxic AhR ligands that possess immunomodulatory properties.

In addition to these classical, synthetic AhR ligands, scientists have begun to discover nonclassical natural ligands. These natural compounds have been shown to be less toxic but still able to elicit responses through the AhR pathway. A majority of natural AhR ligands are introduced into biological systems by oral consumption of foods and herbal medicines, as in the cases of flavonoids, stilbenes, carotenoids, and indoles. Flavonoids include plant products such as quercetin and tangeritin, while resveratrol belongs to a class of stilbenes. Tangeritin, an O-polymethoxylated flavone found in tangerine and other citrus peels, has been shown to lower cholesterol,⁵² inhibit growth of cancer cells,⁵³ and protect against Parkinson's disease.⁵⁴ Indole compounds, such as indole-3-carbinol (I3C) and its chief metabolite 3,3'-diindolylmethane (DIM), are found in cruciferous vegetables, including broccoli, brussels sprouts, cabbage, and cauliflower.⁵⁵ In addition, other dietary products such as carotenoids and curcumin have been found to interact with the AhR. Nutritional

studies have reported that diets rich in carotenoids lead to healthier lives and reduced mortality from a number of chronic illnesses.⁵⁶

Based on the developmental abnormalities exhibited by AhR-deficient mice, researchers began to postulate the existence of endogenous ligands for the AhR. Some of the most basic endogenous ligands are derived from the essential amino acid tryptophan. Tryptophan metabolites such as indolo[3,2-b]carbazole (ICZ), 6-formylindoleo [3,2-b]carbazole (FICZ), and 6,12-diformylindolo[3,2-b]carbazole (dFICZ) bind to AhR and act as immuno-modulators. This was observed based on their abilities to influence cell survival, specifically in various types of cancers. Bilirubin, a heme degradation product, was shown to elevate levels of CYP1A1/2 in Gunn rats and stimulate AhR transformation and XRE/DRE binding in vitro.⁵⁷ Lipids such as prostaglandin PGG2 have recently been shown to competitively displace TCDD from the AhR binding site. Generally, prostaglandins are lipid mediators derived enzymatically from fatty acids and are responsible for regulating the contraction and relaxation of smooth muscle tissue.^{58,59}

Cyclic AMP (cAMP) is a well-known second messenger that mediates hormones, neurotransmitters, and prostaglandins. Recently, it has been shown that cAMP can also activate the AhR. However, unlike the signaling responses initiated by TCDD, the cAMP-dependent form of the AhR undergoes conformational changes that do not require the binding of exogenous ligand for nuclear translocation or the generation of the AhR/ARNT complex.⁶⁰ There is also evidence indicating that some endogenous ligands may actually inhibit AhR activation upon binding. 7-Ketocholesterol, for example, is a major dietary oxysterol that was able to completely block the binding and effects of TCDD.⁶¹

In the search for additional tools to study the role of the AhR in various developmental and disease settings, researchers have turned to exploring small-molecule inhibitors alongside the use of AhR agonists and AhR-deficient mice. α -Naphthoflavone (ANF), for example, is well known for being the classical AhR antagonist. This has been exemplified by its ability to block the effects of TCDD-dependent activation of the XRE-containing reporter gene in Hepa 1c1c7 cells.⁶² However, the antagonistic properties of ANF are limited because ANF can act as a partial agonist of AhR at higher concentrations. In a sense, ANF merely serves as a competitive inhibitor in the presence of another AhR ligand. Recently, more selective AhR ligands have been developed to serve as pure antagonists. 2-methyl-2H-pyrazole-3-carboxylic acid-amide (CH-223191) is a small-molecule inhibitor that appears to act exclusively on the AhR without exhibiting any partial AhR or estrogen receptor agonistic activity. When administered in mice, CH-223191 prevented TCDD-induced liver toxicity, as determined by reduced AST/ALT levels, wasting syndrome, and a lack of CYP1A1 induction.⁶³ Other selective AhR antagonists such as 3'-methoxy-4'-nitroflavone (MNF), 3', 4'-dimethoxyflavone (3',4'-DMF), and 6,2',4'-trimethoxyflavone (6,2',4'-TMF) have shown, in vitro, their ability to prevent TCDD-induced formation of the nuclear AhR complex. In addition, MNF, 3',4'-DMF, and 6,2',4'-TMF were able to block the induction of CYP1A1-dependent ethoxyresorufin O-deethylase (EROD) activity, even though TCDD resulted in a 50- to 80-fold increase in EROD activity.⁶⁴⁻⁶⁶

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

TCDD, commonly referred to as dioxin, is a well-established environmental contaminant that is largely the by-product of combustion, industrial, and natural processes. Traces of TCDD can be found throughout the world in a variety of sources, including the air, water, soil, and sediment. TCDD toxicity has received significant attention in modern history, as TCDD was a contaminant in Agent Orange, an herbicide that was widely used during the Vietnam War. It also garnered significant attention more recently in the 2004 assassination attempt on former Ukrainian president Viktor Yushchenko, whose exposure to TCDD led to chloracne, a skin disfigurement caused by TCDD poisoning. Extensive research on TCDD and its potent toxic and immunosuppressive effects has been ongoing for almost four decades. As alluded to earlier, much of the discoveries surrounding the AhR and its properties were greatly enhanced by TCDD-related research.

Bioavailability

The daily exposure of humans to TCDD occurs mainly through the consumption of food, which can contain small traces of dioxin. Tolerable daily doses of TCDD range from 1–4 pg/kg body weight, as determined by the World Health Organization. On average, humans have been reported to consume around 2 parts per trillion (ppt) on a daily basis.⁶⁷ The half-life of TCDD in the human body also depends on other factors that include age, dose, exposure time, diet, and overall health. Typically, though, the dioxin half-life in adult humans is approximately 7 years, and this number is greatly reduced in infants, down to about 0.4–1.6 years.⁶⁸

Background

Unlike other compounds reviewed here, TCDD is not derived from a natural plant source. Moreover, because this compound is a potent toxin and a potential carcinogen, it cannot be used for therapeutic purposes. However, this compound is in this review for two main reasons. First, the research involving TCDD greatly enhanced and furthered the available knowledge about the AhR. In 1976, Poland et al.⁶⁹ became the first to show that the effects of TCDD were mediated by its interaction with the AhR. Second, research involving TCDD and its interactions with the AhR helped reveal the involvement of this receptor in immunological processes, including immune regulation and suppression. While research has shown TCDD, at very low and nonlethal doses, to have the potential to suppress the immune response, the use of TCDD to treat inflammatory diseases is not feasible because TCDD is also considered a potential human carcinogen. In this regard, the data and research obtained with TCDD and chronicled in this review serve primarily to promote the use of natural AhR ligands that do not possess such toxic effects.

Research on TCDD has shown that this toxin has many effects on the immune system. Some of these effects include thymic atrophy and the suppression of fetal lymphocyte maturation and development.^{3,70} Many review articles on the mechanism of TCDD-mediated immunosuppression have been published in recent years,^{71,72} and therefore such mechanisms will not be further elaborated here. Overall, the mechanisms could be summarized into the ability of TCDD to induce apoptosis in immune cells,^{70,73–76} to alter

cytokines,⁷⁷ and to directly affect various cellular components such as Th cells, cytotoxic T lymphocytes, B cells, thymocytes, and dendritic cells, as detailed in recent reviews.⁷² In addition, TCDD has been shown to induce transforming growth factor- β , which facilitates the generation of suppressor T cells or Foxp3⁺ regulatory T cells (Tregs).^{78,79} Tregs downregulate the immune response of dendritic cells and other T cells through either the secretion of interleukin (IL)-10, transforming growth factor- β , granzymes, IL-2 depletion, or the expression of inhibitory cytotoxic T-lymphocyte antigen 4.⁷² Recent studies suggested that AhR activation promotes epigenetic regulation, thereby influencing reciprocal differentiation of Tregs and T helper 17 (Th17) cells and ameliorating inflammation.⁸⁰

Role of the AhR in the regulation of inflammation and inflammatory diseases

Using TCDD as a potent AhR agonist, many researchers have tested whether AhR activation *in vivo* can suppress inflammation and therefore be used to treat inflammatory and autoimmune diseases. For example, TCDD suppressed allergic and allograft responses.^{81,82} TCDD also prevented graft-versus-host disease⁷⁹ and improved graft survival in an AhR-dependent manner, as evidenced by a constitutively active AhR construct compared with TCDD treatment.⁸³ Researchers also demonstrated how TCDD treatment was able to reduce the inflammation associated with colitis that was induced in mice by the use of 2,4,6-trinitrobenzenesulfonic acid, which resembles the inflammatory response in human Crohn's disease. Upon treatment with 15 $\mu\text{g/kg}$ TCDD, mice recovered body weight faster and showed significantly reduced colonic damage. Proinflammatory cytokines in the gut were also reduced with TCDD treatment.⁸⁴ Recent studies demonstrated that TCDD could suppress the inflammation seen in dextran sodium sulfate (DSS)-induced acute colitis in C57BL/6 mice.⁸⁰ Analysis of T cells in the lamina propria and mesenteric lymph nodes during colitis revealed decreased presence of Tregs and increased induction of Th17 cells, both of which were reversed following TCDD treatment. Interestingly, analysis of mesenteric lymph nodes and lamina propria cells during colitis also revealed increased methylation of CpG islands of Foxp3 and demethylation of IL-17 promoters, both of which were reversed following TCDD treatment. These data, along with the usage of AhR knockout mice, demonstrated that AhR activation promotes epigenetic regulation, thereby influencing reciprocal differentiation of Tregs and Th17 cells, and amelioration of inflammation.

Scientists have also investigated the use of TCDD to study the effect of AhR activation in the treatment of autoimmune diseases. Treatment with TCDD, for example, was able to suppress autoimmune responses in animal models for type I diabetes⁸⁵ and multiple sclerosis.⁸⁶ Studies investigating TCDD treatment of experimental autoimmune encephalitis mice, an animal model used to study multiple sclerosis, showed that TCDD suppressed symptoms of experimental autoimmune encephalitis in an AhR-dependent manner, as exemplified by the use of AhR-dysfunctional (AhR^d) mice.⁸⁶ Furthermore, TCDD was demonstrated to induce functional Tregs, which are a CD4⁺ T cell subset that express Foxp3 and the IL-2 receptor. Interestingly, another AhR ligand, FICZ, showed the opposite effect in this model and increased the severity of the disease. In contrast to TCDD, FICZ interfered with Treg development while simultaneously inducing Th17 cells, which could be inhibited with the AhR antagonist resveratrol. Th17 cells have been reported to play a role in tissue

inflammation via the production of the proinflammatory cytokine IL-17. These data highlight the unique abilities of AhR ligands to yield diverse effects, particularly their ability to induce Tregs versus Th17 cells. Additionally, these studies demonstrate that, once activated, the AhR can regulate immune responses by triggering immunosuppression and can inhibit inflammation by inducing Treg differentiation. However, AhR ligands can also promote inflammation through the generation of Th17 cells, as demonstrated by studies with FICZ.

FLAVONOIDS AND OTHER PHYTOCHEMICALS

Flavonoids such as quercetin and several other phytochemicals have been shown to modulate immune responses through the AhR. The flavonoids are a large class of polyphenolic compounds that are present in a wide variety of fruits, vegetables, and plant-derived beverages such as tea and wine. These compounds are divided into several classes on the basis of their structure, which include flavonols, flavanols, flavans, flavanones, and isoflavones.⁸⁷ Research has shown that flavonoids possess anti-inflammatory, cardiovascular, antiviral, anti-oxidant, and chemopreventive properties.^{88,89} In addition to flavonoids, other phytochemicals, like resveratrol and curcumin, exhibit anti-inflammatory, cytoprotective, and DNA-protective effects.⁹⁰ The low toxicity of these naturally occurring compounds, combined with their variety of effects on the immune system via the interaction with the AhR, makes them intriguing candidates for researchers to investigate. In this review, quercetin will be highlighted as an example of flavonoids, in addition to more well-documented and studied phytochemicals like resveratrol and curcumin.

Quercetin

Background and source—Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavonol that is also one of the most abundant polyphenolic compounds in the human diet.⁹¹ This particular flavonoid can be found in numerous vegetables, fruits, seeds, and nuts, as well as in tea and red wine.⁹² Like many flavonoid compounds, quercetin is biosynthesized through a series of reactions that begin with the essential amino acid phenylalanine. Briefly, phenylalanine is converted to malonyl-CoA through what is termed the “general phenylpropanoid pathway.” Malonyl-CoA is then converted to tetrahydroxychalcone by 7,2'-dihydroxy-4'-methoxyisoflavanol synthase, and this compound is further converted into naringenin by chal-cone isomerase. The formation of eriodictyol and then dihydroquercetin from naringenin leads to the final conversion of quercetin, using flavonol synthase.⁹³

Bioavailability—In many Western societies, the average daily intake of quercetin is estimated to be around 30 mg daily.⁹⁴ Studies in both animal and human models have been performed to address the absorption and metabolism of orally administered quercetin. In rats and pigs given doses ranging from 50 mg/kg to 500 mg/kg, quercetin metabolites were found to be widely distributed in various organ tissues.⁹⁵ In terms of bioavailability, this factor is greatly dependent on which metabolic form is present in the food consumed. For example, it was shown that human peak plasma concentrations of plant-source-derived quercetins (glucose conjugates of quercetin) ranged from 3.5 μ M to 5.0 μ M, whereas unconjugated forms were considerably lower, at less than 0.33 μ M.⁹⁶ In one of the first

human studies concerning the bioavailability of quercetin, 27 healthy humans were given four tablets daily, which contained 250 mg of quercetin, 50 mg of rutin, and 250 mg of other bioflavonoids, for a period of 28 days. After establishing a presupplementation mean plasma concentration of 0.10 μ M quercetin, researchers found that this value increased up to 1.5 μ M after 28 days.⁹⁷ Unlike other well-studied polyphenols, which can have half-lives of up to minutes or only a few hours after oral administration, quercetin is eliminated at a considerably slower rate, with a half-life up to 11–28 h.⁸⁷ Much of quercetin's limited bioavailability is due to its limited solubility in water. This makes it difficult to administer this compound for treatment. However, researchers have been trying to find ways to circumvent this problem. For example, nanoencapsulation of quercetin is currently being investigated as a means of transporting this compound more efficiently to tissues as well as improving efficacy of treatment.⁹⁸

Effect of quercetin on inflammation and inflammatory diseases—Quercetin has been shown in numerous models to elicit anti-inflammatory properties. In mice, quercetin was able to inhibit nitrite oxide and NF- κ B production after challenge with lipopolysaccharide (LPS).⁹⁹ In activated macrophages, quercetin treatment continued to display anti-inflammatory effects by reducing inducible nitric oxide synthase and COX-2 signaling via the suppression of signal transducer and activator of transcription 1 (STAT1) and NF- κ B activation.¹⁰⁰ In polychlorinated-biphenyl-induced inflammation of vascular endothelial cells, quercetin treatment appeared to protect these cells by competitively antagonizing AhR-mediated activation of CYP1A1. In addition, treatment of these cells was able to inhibit the cell-adhesion molecules vascular cell adhesion molecule-1, E-selectin, and P-selectin during polychlorinated-biphenyl-induced vascular inflammation.¹⁰¹ More recently, researchers were able to confirm the ability of quercetin and other plant polyphenols to downregulate the gene expression of known inflammatory mediators, such as IL-8, tumor necrosis factor (TNF)- α , monocyte chemotactic protein 1, IL-6, and COX-2, in LPS-activated cultures with human epidermal keratinocytes.¹⁰² Meanwhile, in LPS-stimulated RAW 264.7 cells, quercetin was shown to reduce phosphorylated extracellular signal-regulated kinase (p-ERK) and p-38 expression, which led to the subsequent suppression of proinflammatory cytokines, such as TNF- α , IL-6, and IL-1 β .¹⁰³ Additionally, quercetin was found to decrease JNK phosphorylation as well as TNF- α and inducible nitric oxide synthase expression in skeletal muscle of *ob/ob* mice and in L6 myotubes treated with palmitate.¹⁰⁴

Curcumin

Background and source—Curcumin is a lipophilic polyphenol found in the rhizome of the plant *Curcuma longa*, which is also known by the common name turmeric when extracted into an herbal powder. *Curcuma longa* belongs to the ginger family Zingiberaceae and is mainly cultivated and distributed in Asiatic regions, such as India and China.¹⁰⁵ Curcumin was first isolated in 1815 by Vogel and Pelletier,¹⁰⁶ and its chemical structure was later confirmed by Milobedzka et al.¹⁰⁷ in 1910. The curcumin found in turmeric has traditionally been used as a spice to add flavor and color to curry.¹⁰⁸ In addition to its use as a spice, curcumin has also been used in traditional Indian medicine (Ayurveda) to treat a number of ailments like anorexia, inflammation, wound healing, arthritis, and sinusitis.¹⁰⁹

Bioavailability—Much like quercetin, curcumin is quickly metabolized and excreted, which thus limits its bioavailability. In experiments involving rats, an intravenous 40 mg/kg dose of curcumin was completely cleared from the plasma within an hour. Following an oral dose of 500 mg/kg in rats, peak plasma concentrations reached only about 1.8 ng/mL.¹¹⁰ The elimination half-life values have also been studied in rats. For these studies, curcumin was given as a 10 mg/kg intravenous dose and a 500 mg/kg oral dose. For the intravenous dose, the elimination half-life values were reported to be about 28.1 h, whereas the oral dose was around 45 h.¹¹¹ Several studies have also been conducted in humans to assess the absorption, metabolism, and bioavailability of curcumin. A onetime oral dose of 10 g or 12 g was administered to healthy subjects, and serum concentrations were determined for up to 72 h. Researchers found that maximum serum concentrations levels were around 2.3 µg/mL for the 10 g dose and 1.73 µg/mL for the 12 g dose. As in other studies, it was found that curcumin was quickly cleared from the serum and seemed to be found more readily, though in low quantities, in the gastrointestinal tract and liver.¹¹² Because curcumin has shown promise in the treatment of a variety of diseases, researchers have tried to find ways use this polyphenol for therapeutic purposes while also trying to counteract or bypass its limited bioavailability. Several techniques have been developed to overcome curcumin's rapid metabolism. For example, 20 mg of an alkaloid called piperine, which is a component of black pepper, was given in combination with 2 g of curcumin to human subjects. Interestingly, the study revealed that serum concentrations of curcumin increased up to 20-fold; this increase in bioavailability was attributed to the inhibition of hepatic glucuronidation and intestinal metabolism.¹¹³ Researchers have also used nanoparticle technology to develop nanocurcumin by synthesizing less than 100 nM of curcumin with copolymers. Other methods using liposomes, phospholipids, micelles, and isomerization are also being developed to enhance the bioavailability of curcumin.¹¹⁴

Effect of curcumin on inflammation and inflammatory diseases—Curcumin has been shown to exert antioxidant, antiproliferative, antiangiogenic, apoptotic, and anti-cancer properties, supporting its use in traditional Indian medicine.¹¹⁵ Studies showed that curcumin was able to protect many tissue types, such as brain, heart, liver, lungs, kidneys, and skin, from oxidative agents. However, much like quercetin, curcumin can also exert pro-oxidative properties if given at high concentrations or in the presence of free iron and copper.¹¹⁶ At the genetic level, curcumin was found to protect DNA against oxidative attack. This protection by curcumin lowers the risk of genetic mutations and other forms of damage to the DNA.¹¹⁷

A wide range of studies have investigated the use of curcumin as an anti-inflammatory agent in both in vitro and in vivo systems. In clinical trials, the use of curcumin has been studied in various inflammatory conditions like ulcerative colitis, eye inflammation, pancreatic inflammation, joint inflammation, and a vast collection of other inflammatory conditions.¹¹⁸ As with other AhR ligands mentioned, curcumin was found to downregulate NF-κB, one of the key nuclear transcription factors involved in regulating acute and chronic inflammatory signaling cascades,¹¹⁹ while also being able to inhibit COX-2 activation.¹²⁰ Other inflammatory mediators shown to be inhibited by curcumin include lipooxygenases,¹²⁰ STAT, peroxisome proliferator-activated receptor γ, and β-catenin.¹²¹ Extensive research

has shown that, in a number of cell types (lymphocytes, monocytes, macrophages, and dendritic cells), curcumin inhibits the proinflammatory cytokines TNF- α , IL-1 β , and IL-8.¹²² Adhesion molecules also play an important role in inflammatory responses, as they bind the T cells to antigen-presenting cells as well as to endothelial cells. In human umbilical vein endothelial cells, pretreatment with curcumin was able to block monocytes from adhering to endothelial cells and was also able to downregulate the expression of other adhesion molecules, like intra-cellular adhesion molecule 1 and vascular cell adhesion molecule 1.¹²³

In addition to the vast research collected on curcumin's anti-inflammatory effects in vitro, researchers have also been able to show this effect in many in vivo animal models. One of the earlier studies of this type included the use of curcumin in a mouse and rat model of carrageenan-induced paw edema. In these studies, 48 mg/kg doses of curcumin were able to reduce the induced edema in mice by at least half, while similar results of decreased edema and inflammation were achieved at even lower doses in rats.¹²⁴ The same researchers were able to show that, at 40 mg/kg doses, curcumin was even able to inhibit formaldehyde-induced arthritis in rats as well. In an experimentally induced mouse model of colitis involving the use of 1,4,6-trinitrobenzene sulfonic acid, pretreatment with a 50 mg/kg dose of curcumin ameliorated neutrophil infiltration and lipid peroxidation in colonic tissue and also reduced damage to the colonic architecture.¹²⁵ In a more recent study, curcumin administration was shown to attenuate the development of allergic airway inflammation.¹²⁶ In these studies, researchers sensitized mice to ovalbumin and challenged them repeatedly with aerosolized ovalbumin. Mice that were treated daily with intraperitoneal injections of 200 mg/kg of curcumin showed decreased inflammation in lungs and reduced numbers of leukocytes and neutrophils in bronchoalveolar lavage fluid. The researchers also noted a decreased level of immunoglobulin E in bronchoalveolar lavage fluid as well. Taken together, these researchers postulated that, by inhibiting NF- κ B activation in asthmatic lung tissue, curcumin can prevent the development of asthma. Although the aforementioned studies demonstrate the anti-inflammatory properties of curcumin, there was no direct evidence linking these effects to AhR signaling.

Resveratrol

Background and source—Resveratrol (3,4',5-trihydroxystilbene) is a naturally occurring nonflavonoid polyphenol that can be found in a variety of dietary sources, including grape seeds, peanuts, and mulberries. As a result of its presence in the seeds and skins of red grapes, this compound can also be found in red wine.¹²⁷ Plants produce resveratrol as a stress response against invading fungus, which highlights the natural antifungal properties of this compound.¹²⁸ This compound exists in both *trans* and *cis* isomer conformations. Research suggests that the *trans* form of resveratrol is the more stable and thus more bioreactive of the two conformations. The *trans* form of resveratrol is converted to the *cis* form in the presence of ultraviolet light and becomes highly unstable at high pH levels. Many studies with this particular compound, along with its various isoforms and chemically modified structures (e.g., glucosylated, prenylated, methylated, and hydroxylated), have reported a host of beneficial effects following resveratrol consumption and absorption.^{129,130}

Bioavailability—Data concerning the bioavailability of resveratrol have been collected in both animal models and human studies. The general consensus from these experiments is that this compound, when taken orally, results in high absorption but undergoes rapid and extensive metabolism. Experiments with [¹⁴C]-*trans*resveratrol in mice and rats revealed that recovery of radioactively labeled resveratrol could be found in the stomach, liver, kidney, intestine, bile, and urine.¹³¹ In particular, high-performance liquid chromatography studies in rats were able to show that absorption of resveratrol peaks in the plasma around 10–15 min after oral administration. Additionally, the rapid elimination of this compound in the plasma resulted in a half-life of about 14 min. It was also shown that about 50–75% of the orally administered resveratrol was absorbed in the aforementioned organ systems.¹³² Studies of resveratrol absorption after oral consumption in humans showed a similar trend, with the plasma half-life being around 9 h and the absorption of a dietary relevant 25 mg dose being 70%.¹³³ A summary of findings on the bioavailability of resveratrol in animal and human studies seemed quite consistent, suggesting that the amount of unchanged resveratrol was very low after consumption, but the rapid and extensive absorption of modified versions of this compound remained in a variety of organ systems.

Effect of resveratrol on inflammation and inflammatory diseases—Research on resveratrol and its potential clinical applications has gained significant attention recently due to the so-called “French paradox.” This phenomenon refers to lower rates of heart disease in the French population despite a high-fat diet, with red wine thought to be the contributing factor.¹²⁹ Resveratrol is an important component of the root of *Polygonum cuspidatum*, also known as ko-jo-kon, which is used in Eastern medicine to treat diseases of the blood vessels, heart, and liver.^{128–134} Resveratrol has also been suggested to have possible therapeutic applications in numerous areas due to its cardioprotective,^{135,136} anticancer,¹³⁷ antioxidant,¹³⁸ cholesterol-lowering,¹³⁹ and antiaging¹⁴⁰ effects.

In antiaging research, the use of resveratrol was found to increase the lifespan of both yeasts and mice.¹⁴⁰ Other research has revealed that resveratrol can be used to treat nonalcoholic fatty liver disease. Resveratrol was capable of modulating a number of genes involved in lipid metabolism in the livers of mice fed an atherogenic diet.¹⁴¹

Past and recent studies reveal resveratrol may be a promising agent to combat inflammatory conditions. In an experimental autoimmune encephalitis mouse model of multiple sclerosis, resveratrol administration was able to reduce the clinical disease score compared with vehicle-treated control mice.¹⁴² Histological data further illustrated resveratrol-treated mice exhibited reduced T-cell infiltration in the spinal cord, which was attributed to the increased number of these cells undergoing apoptosis. These effects were shown to be mediated directly by resveratrol-induced AhR activation through the use of AhR knockout mice and pharmacological inhibitors.

In addition, resveratrol was able to downregulate various proinflammatory cytokines and the number of Th17 cells. Other studies have suggested resveratrol regulates Th17 cells by decreasing the phosphorylated signal transducer and signal transducer and activator of transcription 3 (STAT3). STAT3 is an important regulator of receptor-related orphan

receptor γ , which serves as the first transcription factor to be selectively expressed on Th17 cells.¹⁴³

Further supporting the potential usefulness of resveratrol in inflammatory conditions, research has shown that treatment with resveratrol can modulate the effects of colitis, an autoimmune condition that results in uncontrolled inflammation of the colon and large intestine.¹⁴⁴ Using the DSS model of colitis, research revealed orally administered resveratrol in mice was capable of reducing the weight loss associated with this condition. In addition, resveratrol treatment decreased the number of activated CD4⁺ T cells in mesenteric lymph node and lamina propria tissues. As previously reported, resveratrol was also found to suppress indicators of inflammation such as cytokines TNF- α and INF- γ , Th1 cells, COX-2, and NF- κ B. In addition, mice induced with experimental colitis were found to have decreased expression of silent mating type information regulation-1 (SIRT1) in cells isolated from the lamina propria. Resveratrol treatment was able to counter and increase SIRT1 levels in these cells.¹⁴⁵ A recent study also indicated that resveratrol triggered the induction of immunosuppressive CD11b⁺ Gr-1⁺ cells that were responsible for the suppression of chronic colitis in IL-10^{-/-} mice.¹⁴⁶ Another study investigated the potential use of resveratrol for chronic rhinosinusitis, an inflammatory disease of the nasal and sinus cavities. Using both in vitro and in vivo models, researchers found that resveratrol was not only anti-inflammatory, as demonstrated by the reduction of IL-8, but also a potent promoter of chloride (Cl⁻) secretion in the transepithelial nasal airway.¹⁴⁷ This transport of Cl⁻ in the airway surface liquid of the mucociliary apparatus is important. Disruption of this secretion ability can lead to bacterial infections in the upper and lower airways, which can cause chronic inflammation in the affected area. However, in human keratinocytes activated by a combination of TNF- α and IFN γ , researchers found that resveratrol treatment (50 μ M) led to an increase in IL-8 secretion in an AhR-dependent manner.¹⁰² Collectively, these findings suggest the effects of resveratrol may be dependent on the cell type, the dose administered, and the mode of activation.

TRYPTOPHAN AND ITS DERIVATIVES

Tryptophan, one of the 20 basic amino acids, is the progeny of the fermentation of serine and indole. For many organisms, this essential amino acid cannot be synthesized and must be obtained through dietary uptake. Foods that contain a substantial amount of tryptophan include chocolate, oats, milk, eggs, fish, and poultry. In addition to being a basic building block for protein synthesis, tryptophan serves as a biochemical precursor for the neurotransmitter serotonin,¹⁴⁸ niacin (vitamin B₃),¹⁴⁹ and the phytohormone auxin.¹⁵⁰ Interestingly, several derivatives of tryptophan have shown potential as alternative treatments for various ailments and diseases. 5-Hydroxytryptophan, for example, was used to alleviate epileptic seizures and depression in mice.¹⁵¹ Some tryptophan metabolites have been used for centuries in traditional Chinese remedies in combination with other herbal remedies. Recently, researchers have developed a growing interest in the untapped therapeutic potential of tryptophan and its derivatives. In this portion of the review, a few metabolites will be highlighted, all of which have been shown to have an affinity for the AhR. These metabolites include indigo, a blue dye from the plant *Isatis tinctoria*, and its relatives indigowood root and indirubin as well as I3C and its offspring DIM.

Indigo, indirubin, and indigowood root

Background and source—Indigo is a blue dye isolated from the dried leaves of the flowering plant *Isatis tinctoria*. It has generally been used as an ink and as a dye for fabrics. For centuries, people have used the dried roots of both *Isatis tinctoria* and indigowood to produce elixirs and herbal medicines. Indigowood root has been found to contain many chemical compounds, including indigotin, indirubin, isatin, isaindigotidione, organic acids, and amino acids. Indirubin has also been shown to be an active component of the traditional Chinese herbal formula Danggui Longhui Wan, a mixture of 11 herbal extracts used in the treatment of chronic diseases. Typically, indirubin is offered as an oral tablet at concentrations of 150–200 mg.¹⁵²

Bioavailability—After intraperitoneal administration of 5.6 mg/kg in rats, indirubin displayed a relatively low bio-availability of 8.4%.^{153,154} Consequently, indirubin appears to have a significantly lower half-life in rats (about 1 h) than in mice (17.5 h). Indigo and indirubin have also been reported to be present at low levels in human urine. As a result, researchers have spent more than two decades trying to design indirubin derivatives to overcome the poor solubility and absorption of indirubin and to improve its pharmacological effects.

Effect of indigoids on inflammation and inflammatory diseases—Initial studies in yeast suggested indigoids may serve as physiological ligands for the AhR. In those studies, indigo and indirubin were found to be 50 times more potent than TCDD.¹⁵⁵ In mammalian systems, however, endogenous levels of indirubin (picomolar and nanomolar) only lead to transient translocation of the AhR to the nucleus and do not result in DNA binding.¹⁵⁶ Studies using a hepatoma cell reporter system indicated the half-maximal effective concentration (EC₅₀) values for indigo and indirubin 3'-oxime are probably closer to 5×10^{-6} M and 5×10^{-7} M, respectively.⁴² Other studies have shown that only with these higher doses (micromolar) does indirubin exhibit a profile similar to that of TCDD in which it generates the formation of the AhR/ARNT dimer and significantly induces CYP1A1. In addition, higher doses of indirubin were able to modulate the cell cycle and proliferation of liver progenitor cells or hepatoma cells.¹⁵⁶ These findings suggest that, even though indigo and indirubin appear to be less potent than classic halogenated dibenzofurans or dibenzo-p-dioxins like TCDD, they can still manage to mediate responses through the AhR.

Indigo and indirubin are known to be active components in the traditional Chinese remedy Danggui Longhui Wan, a mixture of 11 herbal extracts that is commonly used to treat chronic diseases such as chronic myelogenous leukemia. Clinical trials have also indicated the effectiveness of indirubin in treating chronic myelogenous leukemia, partially due to its general bone-marrow suppressing action.¹⁵⁷ Indigowood root has been used in traditional medicine for its potential anti-inflammatory and antiviral effects; in addition, it is used to reduce fever and to reduce the occurrence and severity of convulsions. The use of indigowood root in traditional medicine prompted researchers to investigate the therapeutic properties of this herb. In a study to determine the radioprotective effects of indigowood root, mice were exposed to whole-body irradiation with 1.8 Gy/day at a dose rate of approximately 300 cGy/min for 3 consecutive days before treatment. Indigowood root

treatment helped the irradiated mice recover from leukocytopenia, defined as reduced counts of granulocytes, monocytes, and lymphocytes, as well as from weight loss observed in the spleen and thymus. It also significantly reduced serum TNF- α , IL-1 β , and IL-6 while increasing serum granulocyte-colony stimulating factor (G-CSF). Histology revealed indigowood root treatment also reduced intestinal toxicity.¹⁵⁸

Indole-3-carbinol

Background and source—Dietary indole compounds have been used for centuries in traditional Chinese medicine to treat a variety of ailments and diseases. Two indoles that have attracted recent attention for their therapeutic potential are I3C and DIM. I3C is a metabolite of glucosinolate glucobrassicin, which is a chief component of cruciferous vegetables such as broccoli, cauliflower, and brussels sprouts.⁵⁵ When digested, I3C undergoes acid hydrolysis within the gut and is converted to a series of by-products, the major one being DIM.¹⁵⁹

Bioavailability—High-performance liquid chromatography has been used in pharmacokinetic studies in mice to determine the bioavailability of I3C and DIM in various tissues, including plasma, liver, kidney, lung, heart, and brain. Results showed that I3C and DIM were rapidly absorbed and present in all tissues following oral administration. Tissue analysis indicated concentrations ranged from 1 μ M to 500 μ M within 30–60 min before exponentially decreasing. Liver tissue appeared to contain the highest concentration of indoles, while brain tissue and plasma contained the quantifiably lowest concentrations.¹⁶⁰ Additional studies in humans have suggested that the bioavailability of I3C and DIM are similar to that seen in mice.^{160,161} The estimated half-life for these compounds has been suggested to range 12–24 h before being completely metabolized and/or excreted.¹⁶² In addition, both I3C and DIM are currently available as dietary supplements in a multitude of different formulations and dosages.

Effect of I3C and DIM on inflammation and inflammatory diseases—One of the foremost reasons dietary indoles, specifically I3C and DIM, have become so popular among researchers and clinicians is because of their chemopreventive characteristics. These natural compounds have been shown to be effective in suppressing carcinogen-induced and spontaneous tumors in an assortment of tissues, including mammary glands,^{163,164} liver,¹⁶⁵ prostate,¹⁶⁶ tongue and nasal mucosa,¹⁶⁷ and endometrium.¹⁶⁸ The mechanisms underlying the chemotherapeutic properties of I3C and DIM are context dependent; i.e., the effects observed are determined not only by the cell types involved but also by the activation status of the cells.

Studies have shown the therapeutic potential of both I3C and DIM may lie in the fact that they are weak ligands for the AhR. These indoles stimulate cytochrome P450 activity through the AhR, which leads to the generation of phase I and phase II carcinogen-metabolizing and detoxification enzymes such as CYP1A1 and CYP1A2.^{169,170} In turn, I3C and DIM are able to regulate cancer cell growth in a variety of ways, one of which is to regulate estrogen metabolism, thereby reducing the risk of certain hormone-driven cancers. For example, when levels of 16 α -hydroxyestrone are greatly elevated, a shift in the 2:16-

hydroxyestradiol ratio is created, which has been correlated with the risk of developing breast cancer.¹⁷¹ In clinical trials, I3C and DIM have been shown to increase the 2:16-hydroxyestradiol ratio, thus creating an antiestrogenic and antiproliferative environment. These events may in part be attributed to indole-mediated AhR activation, leading to elevated levels of cytochrome P450 isozymes, which are essential for the oxidative metabolism of estrogens. Subsequently, these isozymes increase estradiol 2-hydroxylation activity, resulting in a reduced risk of breast cancer.^{163,164,172,173}

Studies indicating the anticancer properties of dietary indoles generated increased interest in these compounds, and scientists began exploring the effects of I3C and DIM in diseases other than cancer. During this journey, I3C and DIM have been shown to interact with numerous nuclear receptors such as the AhR, the estrogen receptor,¹⁷⁴ and the androgen receptor,¹⁷⁵ and have also been shown to modulate multiple signaling pathways such as phosphoinositide 3 kinase, Akt, and MAPK.¹⁷⁶ In addition, it is possible that these dietary indoles may regulate molecular and cellular pathways through receptor crosstalk, independent of certain downstream mediators such as CYP1A1.¹⁷⁶

Recent studies have indicated that these compounds may play a critical role in modulating immune responses, particularly with regards to inflammation and inflammatory diseases.^{177,178} Systemic lupus erythematosus (SLE), for example, is an autoimmune disease in which auto-antibodies and self-reactive T cells are generated and directed at host tissue. In addition, cytokines such as TNF- α and IL-6 and other proinflammatory mediators create an inflammatory environment that results in severe systemic organ damage.¹⁷⁹ Auburn et al.¹⁸⁰ reported 80% of SLE mice given I3C-supplemented diets survived, compared with only 10% of control mice. While mice from all groups developed anti-double-stranded DNA antibodies, I3C-fed mice presented significantly lower levels. In addition, renal disease as measured by proteinuria, histological changes, immunoglobulin G immune complex deposition, subepithelial deposits, and diffuse epithelial cell foot process effacement was less severe in I3C-fed mice.

Dietary indoles have also demonstrated their anti-inflammatory properties by reducing the generation of reactive oxygen species from murine peritoneal macrophages and LPS-activated macrophages.¹⁸¹ This effect was primarily due to indole-mediated inhibition of I κ B α phosphorylation, which prevents NF- κ B from translocating into the nucleus and generating additional inflammatory mediators.¹⁷⁶ In a DSS-induced colitis model, DIM was able to hinder the weight loss, colon shortening, and severe clinical signs that were observed in diseased mice.¹⁸²

Along with modulating immune responses in autoimmune diseases, indoles also seem to play a role in controlling viral infection. Indole-treated mice exhibited an enhanced host response to enteric reovirus infection.¹⁸¹ Following oral administration of DIM, mice infected with reovirus serotype 1 showed increased levels of G-CSF, IL-6, IL-12, and IFN- γ . These cytokines play a critical role as mediators in the host response to infection. IL-6, for example, serves as a growth factor to T cells and a differentiation factor in B cells.^{183–186} Meanwhile, G-CSF, IFN γ , and IL-12 have all been reported to promote antimicrobial and antiviral activities^{187–189} as well as to control inflammation.^{177,178}

CONCLUSION

Evidence gathered by researchers over the years, based primarily on AhR activation using TCDD, has shown the importance of the AhR in biological systems. Furthermore, the AhR and its ligands may serve as tools for modulating immune function. As highlighted in this review, there are some AhR ligands that can exert anti-inflammatory effects, while others may act in a more proinflammatory manner (e.g., FICZ). These paradoxical observations can be attributed to a variety of factors, such as cell type, response to certain external stimuli, and agonistic/antagonistic nature of the AhR. More recently, natural AhR ligands have also been shown to regulate a number of cellular pathways and biological processes. The mechanisms by which these compounds execute their effects are multifaceted, ranging from receptor signaling to regulation of cell growth and survival to modulation of immune functions and inflammatory mediators. In fact, due to their diverse properties and inconsequential side effects, resveratrol, I3C, and DIM are currently being used in clinical trials to treat diabetes,¹⁹⁰ Alzheimer's disease,¹⁹¹ SLE,¹⁹² human papillomavirus infection,^{193,194} and various forms of cancer.^{195–197}

The precise mechanisms through which natural ligands of the AhR modulate the immune functions by inducing apoptosis in activated T cells¹⁴² or by altering the T cell differentiation from proinflammatory Th17 to immunosuppressive Tregs⁸⁰ need to be further explored so that better AhR ligands with anti-inflammatory properties and minimal toxicity to other organ systems can be developed. In addition, the therapeutic effects observed by use of many of these ligands still need to be further corroborated to be directly related to their involvement with the AhR. This review provides findings that connect the anti-inflammatory effects of some AhR ligands (TCDD, resveratrol, quercetin, I3C, DIM) with the ability of these ligands to interact with the AhR. However, while other ligands such as curcumin have also been proven to demonstrate anti-inflammatory and therapeutic effects, the direct role of the AhR in these effects has yet to be defined. Recent studies indicating that AhR activation leads epigenetic regulation of immune response genes open up new avenues to explore the mode of action of dietary AhR ligands.^{198–200} Altogether, studies have established that natural AhR ligands not only play a vital role in maintaining bio-physiological homeostasis but may also have potential as novel therapeutic agents for managing a wide range of inflammatory conditions and diseases.

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Figure 1. AhR domain structure

Domains: bHLH, basic helix-loop-helix; PAS, Per/Ahr-ARNT/Sim; glutamine-rich region.

Figure 2. AhR signaling pathway

(a) Ligand passes through plasma membrane into the cellular cytoplasm. (b) Ligand binds the cytosolic AhR complex. (c) The ligand-bound AhR complex is translocated into the nucleus. (d) The ligand-bound AhR complex dissociates from chaperone proteins after dimerization with ARNT. (e) Ligand-bound AhR/ARNT complex binds to DREs/XREs, (f) which leads to transcriptional activation of target genes.

Structure & Name	Binding Affinity	Structure & Name	Binding Affinity
2,3,7,8-Tetrachlorodibenzo(p)dioxin (TCDD)	1 pM – 10 nM ³⁹⁻⁴⁰	Quercetin	1 – 20 μ M ^{44, 46}
6-formylindolo[3,2-b]carbazole (FICZ)	30 pM – 830 pM ^{10, 41}	Resveratrol (RES)	1 – 50 μ M ^{44, 47}
Indirubin	1 – 100 nM ⁴²⁻⁴³	3,3'-diindolylmethane (DIM)	80 nM – 100 μ M ⁴⁸⁻⁵⁰
Curcumin (keto form)	1 – 20 μ M ⁴⁴⁻⁴⁵	Indole-3-carbinol (I3C)	0.1 μ M – 200 μ M ⁵⁰⁻⁵¹

Figure 3. AhR ligand structure and binding affinities

Binding affinities are based on half-maximal effective concentration (EC_{50}) values as determined by induction or inhibition of CYP1A1 expression.