CHAPTER 19

Aryl Hydrocarbon Receptor (AhR) Activation: An Emerging Immunology Target?

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Contents

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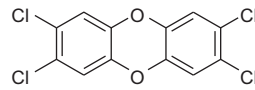
ABBREVIATIONS

AhR	aryl hydrocarbon receptor
ARNT	aryl hydrocarbon nuclear translocator
BaP	benzo[*a*]pyrene
bHLH	basic helix-loop-helix
BMDCs	bone marrow-derived dendritic cells
cDNA	complementary deoxyribonucleic acid
CYP	cytochrome P450
DCs	dendritic cells
DNA	deoxyribonucleic acid
DRE	dioxin response element
EAE	experimental autoimmune encephalomyelitis
ER	estrogen receptor
FICZ	6-formylindolo[3,2-*b*]carbazole
GR	glucocorticoid receptor
H/W	Hans–Wistar
hsp	heat shock protein
IC_{50}	half maximal inhibitory concentration
IDO	indoleamine dioxygenase
IL	interleukin
ITE	2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester
L/E	Long–Evans
LPS	lipopolysaccharide
mRNA	messenger ribonucleic acid
MS	multiple sclerosis
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHR	nuclear hormone receptor
NLS	nuclear localization sequence
NOD	non-obese diabetic
PAH	polycyclic aromatic hydrocarbon
PAS	period clock, aryl hydrocarbon nuclear translocator, and single-minded
PGE_2	prostaglandin E_2
RNA	ribonucleic acid
ROR-γt	retinoic acid receptor-related orphan receptor-γt
Saa	serum amyloid A
SAhRM	selective aryl hydrocarbon receptor modulator
TAD	transcriptional activation domain
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TGF-β	transforming growth factor-β

TNF-α	tumor necrosis factor-α
Treg	regulatory T cell
XRE	xenobiotic response element

1. INTRODUCTION

The aryl hydrocarbon receptor (AhR) was originally characterized as a nanomolar-affinity receptor for the polychlorinated aromatic species dioxin (**1**; also called TCDD for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin), responsible for the toxic effects—such as thymic involution and tumor promotion—observed in animals following exposure to this industrial pollutant. This fashioned the view that the primary role of the AhR was in sensing and orchestrating the response to xenobiotic exposure. However, within the past decade, this view has dramatically changed. This review will highlight research on the key role that the AhR plays in the immune system.



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2. OVERVIEW OF THE AhR

2.1. Description

The AhR is a ligand-activated transcription factor that resides in the cytoplasm in its unactivated state. The N-terminal portion contains a nuclear localization signal (NLS), a bHLH (basic helix-loop-helix) domain, and two PAS (period clock, aryl hydrocarbon nuclear translocator, and single-minded) subdomains. These regions are necessary for heterodimerization with the aryl hydrocarbon nuclear translocator (ARNT) and DNA binding (*via* the bHLH region). The ligand binding site spans the more C-terminal of the two PAS subdomains. The C-terminal motif contains the transcriptional activation domain (TAD) [1].

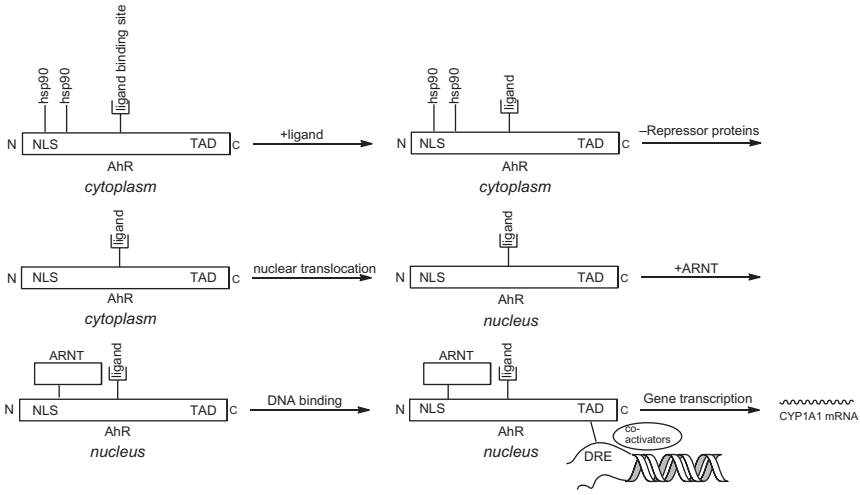
Constitutive tissue expression in adults generally appears to be highest in the liver, with significant expression also in the lung, placenta, thymus, and cornea [2–4]. Induced expression of the receptor has been reported in several types of immune cells when they are exposed to particular stimuli (see below for examples).

Although the AhR belongs to the bHLH and not the nuclear hormone receptor (NHR) superfamily, its canonical signaling pathway (see below) is reminiscent of that for type 1 NHRs, such as the glucocorticoid and estrogen receptors (GR and ER, respectively) [5]. The AhR is an orphan

receptor, since an endogenous agonist has not yet been conclusively demonstrated.

2.2. Canonical signaling pathway

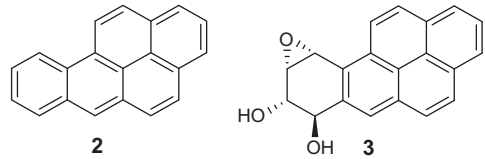
The AhR in its resting state is a cytoplasmic complex with the repressor proteins hsp90, p23 (also known as prostaglandin E synthase 3), and hepatitis B virus X-associated protein 2. The canonical signaling pathway begins with agonist binding to the receptor, which causes a conformational change in the protein that induces dissociation of the repressor proteins. Subsequent unmasking of an NLS is followed by nuclear translocation and heterodimerization with ARNT to form the active transcription factor. The ligated AhR–ARNT complex binds to a consensus nucleotide sequence containing a core recognition element, which has been dubbed the dioxin response element (DRE), or, alternatively, the xenobiotic response element (XRE). This nomenclature reflects the initial characterization of the AhR as the cognate endogenous dioxin receptor, which directs the response to xenobiotic challenge. Up- or downregulation of protein production follows, depending on whether the DRE is contained within the associated gene promoter or repressor [1].



AhR canonical signaling pathway schematic

The canonical signaling result is upregulation of the cytochrome P450 (CYP) enzyme subtypes CYP1A1 and CYP1B1 [6]. Dioxin itself is a poor substrate for enzymatic oxidation, as evidenced by plasma half-lives of weeks (in rodents) to years (in humans) following systemic exposure [7]. Dioxin biopersistence thus leads to sustained CYP enzyme expression elevation, which may lead to tumor promotion through the constant

generation of DNA-damaging reactive oxygen species and electrophilic compounds. Additionally, selective AhR agonists of the polycyclic aromatic hydrocarbon (PAH) class are themselves transformed into DNA-damaging species *via* CYP-catalyzed oxidation, as for example in the conversion of benzo[*a*]pyrene (BaP; **2**) into the mutagenic epoxide **3**.



2 **3**

2.3. Noncanonical signaling pathways

The most immunologically significant noncanonical signaling pathway involves the NF-kB protein family. Physical interaction has been observed between the AhR and members of the NF-kB family, such as RelB, RelA/p65, and p50 [8–10], and different consequences of these interactions have been reported. Interestingly, a binding site in the promoter region of the *Il-8* gene for the AhR/RelB complex has been described, with dioxin causing upregulation of IL-8 protein in macrophages [8]. This implies that NF-kB-linked noncanonical activation is proinflammatory.

In contrast, dioxin and several other AhR agonists inhibited cytokine-induced transcription of the acute phase response genes *Saa1-3* in several cell types [11]. This effect was dependent on AhR nuclear translocation and complexation with ARNT but not DRE binding, thus implicating noncanonical signaling. Furthermore, AhR-knockout mice were more susceptible to lipopolysaccharide (LPS)-induced lethality and cigarette smoke-induced lung neutrophil influx, with coincident loss of nuclear RelB in lung cells and increase in macrophage production of TNF-α and IL-6. Thus, the literature in general suggests that activation of noncanonical NF-κB-linked signaling is anti-inflammatory [12,13].

2.4. Pharmacology tools

The isolation of soluble rodent AhR protein is typically accomplished from the liver, which is the richest source tissue. Use of this technique in transgenic mice with conditional, liver-specific protein knock-in, has been reported for human AhR isolation [14]. The placenta has also been used as an alternative source for the human receptor [15].

Alternatively, a robust procedure for routine scalable isolation of soluble rat and human receptor consists of cloning human or rat AhR cDNA into recombinant baculovirus, followed by infection of insect (*Spodoptera frugiperda*) cells. Cell lysis followed by standard cytosol isolation

affords protein with a 20-fold higher ligand binding capacity compared to standard techniques [16].

2.4.1. Receptor binding and functional activation

Receptor binding assays have commonly used [^{3}H]-dioxin (for scintillation-based detection) or the photoaffinity ligand 2-azido-3-[^{125}I]-7,8-dibromodibenzo-*p*-dioxin (for autoradiography-based detection) as the high-affinity radioactive ligand [16,17]. Receptor functional activation frequently has been measured in an electrophoretic mobility shift assay detecting the binding of the AhR–ARNT heterodimer with a DRE-containing oligonucleotide, or by measuring upregulation of CYP1A1 mRNA or protein expression [18]. A higher-throughput method for detecting receptor activation comprises measurement of luciferase activity in a cell line containing a DRE-driven luciferase reporter gene, after test agent treatment [19].

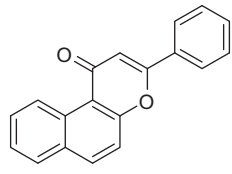
2.4.2. Benchmark ligands

For historical reasons and due to its high binding affinity, dioxin has been the key comparator agonist since the initial characterization of the receptor. A large number of toxicology studies in rodents, and a smaller but burgeoning number of reports from more discrete rodent disease models, have used dioxin in this role.

However, this presents at least two problems. First, dioxin's exceptionally long half-life implies sustained pharmacodynamics far in excess of other well-characterized signaling systems. This limits the extrapolation of these results to the effects of shorter-lived agonists, especially potential endogenous ligands.

Second, it is well established that the receptor binding affinity of dioxin varies substantially between humans and mice and within different mouse strains. Thus, the routinely used C57/Bl6 mouse strain harbors an AhR with a 10-fold higher dioxin binding affinity than that for the human and the DBA/2 mouse strain receptor [17,20]. Dioxin *in vivo* effects are also strain dependent for both mice and rats (see below).

β-Naphtoflavone (**4**) and the tryptophan photoproduct 6-formylindolo [3,2-*b*]carbazole (FICZ; **5**) are reported dioxin-alternative comparator AhR agonists [21,22]. Their main advantage is an increased metabolic liability leading to a shorter plasma half-life. Consequently, their pharmacodynamic effects are more easily interpreted. Also, FICZ is a candidate endogenous AhR agonist, as it is formed in a pharmacologically-relevant concentration in skin. Their main disadvantage is a much smaller database of published studies.



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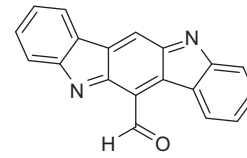

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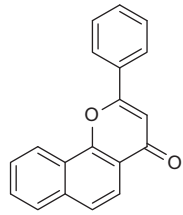
Although there are a number of small molecules claimed in the literature to be AhR antagonists, only a few have been commonly used as pharmacology tools, with α-naphthoflavone (**6**) being a popular example [23]. The pyrazole **CH-223191** (**7**) has emerged as an alternative, based on its potent antagonism of dioxin-induced AhR signaling in mice *in vitro* and *in vivo* [24]. Unlike most other reported antagonists, **7** has no detectable agonism at either the AhR or the ER. However, **7** does not antagonize signaling by β-naphthoflavone [25].



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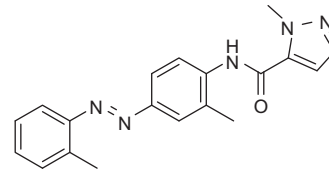

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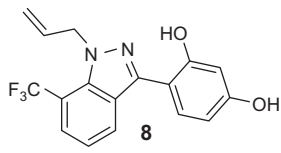
Analogous to other hormone receptors such as the GR and ER, there exists for the AhR a third class of ligands that elicit a subset of agonist effects reported for dioxin *in vitro*. Thus, the indazoles **WAY169916** (**8**) (itself a pathway-selective ERα agonist) and **SGA360** (**9**) and the α-naphthoflavone analog **10** all displayed submicromolar binding affinity to the human AhR. These compounds antagonized dioxin-induced AhR-dependent effects *in vitro* (such as *Cyp1a1* message upregulation) yet exhibited AhR-dependent anti-inflammatory effects *in vitro* and *in vivo* in mice [26–28]. These have been dubbed selective AhR modulators (SAhRMs).



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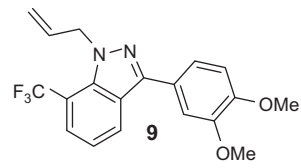

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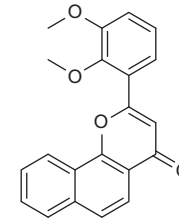

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2.4.3. Rodent AhR

Wild-type mice can be separated into two groups: those with high ($IC_{50} \leq 2$ nM) and those with low ($IC_{50} \geq 20$ nM) dioxin binding affinities for their respective AhRs [20]. High-affinity strains are classified as having AhR^b alleles, while low-affinity strains are designated as having AhR^d alleles. The former group includes the C57/Bl6 and Balb/c strains, while the latter group includes the DBA/2 and non-obese diabetic (NOD) strains. The human AhR is more similar to the murine AhR^d (*i.e.*, lower affinity) allele. However, even between these two proteins, there are significant differences in binding affinities for other AhR ligands [17]. Additionally, dioxin gene expression effects were substantially different in hepatocytes from wild-type C57/Bl6 mice versus those from mice with liver-specific human AhR knock-in [29].

In rats, different susceptibilities to dioxin toxicity are well documented. In particular, an at least ~1000-fold increased dioxin LD_{50} dose was observed for the Hans–Wistar (H/W) versus the Long–Evans (L/E) rat. Although the AhR for the resistant H/W strain has a ~40 amino acid deletion in its TAD compared to the L/E strain, no difference was observed between the two strains in either dioxin binding affinity or dioxin potency and efficacy in hepatocyte *Cyp1a1* message and enzymatic activity induction [30].

AhR-knockout mice have been the most well studied. Although the specific phenotype depends on the animal genetic background and the method of knockout generation, widespread observations include an expected loss of sensitivity to dioxin toxicity, development of several anatomic abnormalities such as increased liver fibrosis, and reduced fertility [1]. Immunologic-based phenotypic dysfunction observed in these animals (besides the increased inflammatory response to LPS and cigarette smoke mentioned earlier) includes increased lethality upon *Toxoplasma gondii* infection [31]. Most of the effects of AhR knockout on cell function have been described in immune cells, as discussed below.

Other AhR mutations useful for analyzing the contribution of the discrete sequences of AhR signaling include the $AhR^{nls/nls}$ genotype, in which the protein lacks a functional NLS, and the $AhR^{dbd/dbd}$ genotype, where the mutant AhR–ARNT dimer is unable to recognize DRE-containing DNA [32].

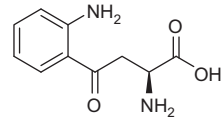
3. EFFECTS ON IMMUNE CELL FUNCTION

3.1. Dendritic cells

Deactivation of dendritic cells (DCs) plays an important role in the immunosuppressive effects of dioxin. There are several mechanisms by which this occurs. Dioxin activated naïve murine splenic DCs *in vitro*,

increased expression of co-stimulatory molecules like CD40 and proinflammatory cytokines like IL-12, and induced proliferation of cocultured allogenic T cells. This antigen-independent activation caused DC death, as evidenced by a lower splenic DC count in dioxin-treated mice [33].

In contrast, LPS upregulated AhR protein expression in bone marrow-derived DCs (BMDCs) *in vitro*, affording a several hundred-fold increase in the secretion of the anti-inflammatory cytokine IL-10. *In vitro*, this effect which was greatly diminished in $AhR^{-/-}$-derived BMDCs [34]. Additionally, dioxin upregulated the enzyme indoleamine dioxgenase (IDO) in DCs [35]. IDO catalyzes the biosynthesis of kynurenine (**11**) *via* oxidation of tryptophan and has long been known to be immunosuppressive. In a further twist, kynurenine itself was characterized as an AhR agonist in murine hepatocytes, with a potency consistent with its physiological concentration [36]. Thus kynurenine may function endogenously as both a downstream and an upstream effector of AhR activation-induced, DC-mediated immune tolerance.



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Furthermore, AhR activation-induced, DC-mediated immunosuppression likely occurs largely *via* DC-evoked conversion of naïve T cells to regulatory T cells (Tregs), a deactivated phenotype important in immune tolerance, as discussed below. For example, adoptive transfer of AhR agonist-treated, antigen-stimulated DCs induced $CD4^+Foxp3^+$ Tregs in host animals and reduced pathology in animal autoimmune disease models [37,38].

3.2. $CD4^+$ T cells

Research to date on the effects of AhR activation on T cell function has concentrated on naïve $CD4^+$ T cell differentiation to either T_H17 cells or Tregs. T_H17 cells are important in host defense against extracellular microorganisms and are important in the pathology of several autoimmune diseases. They are defined by their expression of the transcription factor retinoic acid receptor-related orphan receptor-γt (ROR-γt), are characterized by their secretion of the cytokines IL-17A and IL-22, and in mice, are induced by treatment of naïve $CD4^+$ T cells with TGF-β + IL-6.

$CD4^+$ Tregs are important in maintaining tolerance to self-antigens. The most commonly used molecular biomarker is Foxp3 protein expression. However, Treg definition is based more on phenotypic behavior,

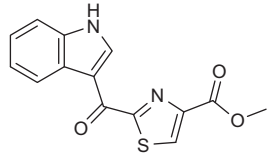
such as anergy to antigen stimulation and deactivation of neighboring effector T cells, than on Foxp3 expression. They are classically generated by treatment of naïve $CD4^+$ T cells with TGF-β.

Thus these two T cell types have opposite functions. An increased T_H17/Treg ratio from homeostatic values can lead to loss of immune self-tolerance and is implicated as a causative factor in several autoimmune diseases.

There is some uncertainty in the literature regarding whether there is a general effect, and if so in which direction, of AhR activation on T_H17/Treg differentiation. Part of this confusion may be due to definitions of protein expression in these cell types that are not absolute. For example, a population of murine $CD4^+$ T cells secreting substantial IL-17A but not expressing ROR-γt has been characterized [39]. $CD4^+Foxp3^-$, dioxin-inducible functional Tregs have been described in mice and humans [40,41]. Moreover in human $CD4^+$ T cells, Foxp3 expression below a threshold level was insufficient for Treg function [42].

However, the main reason for the controversy is that in mice, the alternative AhR agonist FICZ has been reported to polarize naïve $CD4^+$ T cells to a population with a high T_H17/Treg ratio *in vitro*, while the classical agonist dioxin has been reported to produce the opposite effect. In support of the T_H17-inducing hypothesis, AhR protein expression was upregulated by several hundred fold when naïve murine $CD4^+$ T cells were exposed to T_H17-polarizing conditions [43,44]. Murine Tregs exhibited a much lower relative expression of the AhR. Differentiation of murine naïve T cells to T_H17 cells *in vitro* was inhibited unless natural AhR agonists like tryptophan were present in culture [45]. The anomalous result with dioxin is typically attributed to an endogenously irrelevant, sustained AhR activation due to dioxin biopersistence. Alternatively, dioxin may selectively kill T_H17 cells (which express the highest AhR levels for any helper T cell subset) *via* receptor overstimulation, by analogy to dioxin-induced DC dropout mentioned earlier [46].

In support of a general T_H17-inhibiting/Treg-inducing effect of AhR activation, a number of AhR agonists besides dioxin, such as kynurenine, tryptophan-derived 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE; **12**), and the aminopyrimidine **VAF347** (**13**), induced Treg polarization *in vitro* [36–38]. Differentiation of TGF-β-treated naïve murine $CD4^+$ T cells to Foxp3+ Tregs was depressed up to 70% in AhR-knockout cells [36]. Knockdown of *Ahr* message by siRNA inhibited *Il-10* message by 50% in a murine $Foxp3^-$, IL-10-secreting Treg population [47]. In contrast to its effects in murine T cells, FICZ inhibited human T_H17 cell generation *in vitro* [48]. Thus the literature on balance suggests that AhR activation induces Treg phenotype and immune tolerance in humans; however, the general applicability of this hypothesis awaits confirmation with a wider variety of agonists.



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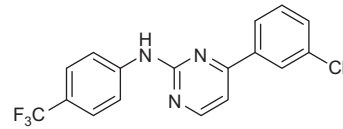

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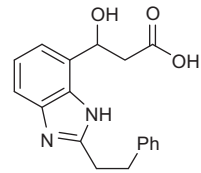
With respect to other murine $CD4^+$ T helper cell subtypes, polarization to T_H2 cells was inhibited in an AhR-dependent manner by the benzimidazole **M50354** (**14**) *via* suppression of the T_H2 master transcription factor *Gata-3* mRNA. T_H2 polarization was also inhibited by expression of a constitutively active AhR in naïve $CD4^+$ T cells [49].



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4. THERAPEUTIC EFFECTS IN ANIMAL MODELS OF HUMAN DISEASES

4.1. Experimental autoimmune encephalomyelitis (EAE)

Multiple sclerosis (MS) is a chronic, usually progressive neurological disease with symptoms including abnormal sensations, muscle weakness, and paralysis. Disease pathology is believed to be due to attack of effector T cells and macrophages on cells that form the myelin sheath of myelinated nerves in the central nervous system. The standard of care during acute attacks is treatment with immunosuppressants, such as steroids and interferon-β-1. Consensus opinion is that these treatments are only palliative.

Rodent EAE is a widely-used model of human MS and is typically induced by immunization with a myelin oligodendrocyte glycoprotein. Disease progression from minimal locomotor effects to complete paralysis is represented by increased numerical scores. Of the three AhR agonists with published data in murine EAE models, ITE and dioxin decreased, while FICZ increased, disease scores [37,43,44]. Dioxin and ITE therapeutic effects were not observed in animals with the AhR^d (low affinity) allele. In the absence of agent treatment, these mice also exhibited exacerbated pathology compared to AhR^b (high affinity) animals [37,44].

4.2. Colitis

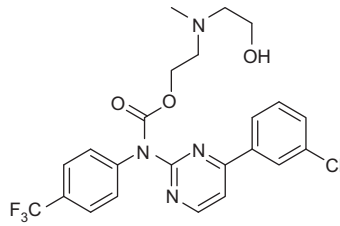
Colitis (inflammation of the bowel) due to Crohn's disease is characterized by chronic diarrhea and abdominal pain. Affected patients are at increased risk of malnutrition due to nutrient malabsorption, and intestinal cancer due to persistent inflammation. The disease is thought to be caused by autoimmune attack on the gastrointestinal tract and is typified histologically by neutrophil infiltration into and granuloma formation within the bowel. Similar to the situation with MS, the standard of care is treatment with anti-inflammatory/immunosuppressant agents, such as steroids and TNF-α blockers.

Colitis is typically induced experimentally in mice by oral treatment with dextran sodium sulfate or 2,4,6-trinitrobenezenesulfonic acid. Observed effects of clinical relevance to humans include weight loss and colonic ulceration. In mouse models of colitis, dioxin inhibited weight loss and decreased colon damage with concomitant reduction in colonic TNF-α and increase in cytoprotective PGE_2 and $Foxp3^+$ Tregs [50,51]. Seemingly contradictory, in another disclosure dextran sodium sulfate-treated, $AhR^{+/-}$ mice had reduced damage and decreased colonic TNF-α expression compared to wild-type controls, while human inflammatory bowel disease patients had higher colonic AhR expression compared with normals [52]. Thus the effect of AhR activation on inflammatory bowel disease pathology awaits clarification *via* further research.

4.3. Allergic asthma

Asthma is an airway disease in which abnormal bronchial constriction and mucus hypersecretion lead to insufficient oxygen reaching the lungs. Acute attacks are often triggered by exposure to an allergen. The standard of care for acute attacks is inhalation of a β-adrenoceptor agonist like albuterol (or in emergency situations, injection of the endogenous β-adrenoceptor agonist epinephrine). Management of chronic disease is typically achieved with a combination of a long-acting β-adrenoceptor agonist as a bronchial smooth muscle relaxant and a glucocorticoid as an anti-inflammatory. Reducing lung influx of professional inflammatory cells, especially eosinophils, is likely an important effect of the anti-inflammatory component.

In a Balb/c mouse model of ovalbumin-induced allergic asthma, prodrugs of the aforementioned AhR agonists **13** and **14**, namely **VAG539** (**15**) and **M50367** (**16**), reduced several pathological hallmarks, such as serum IgE levels, acetylcholine-induced bronchoconstriction, and pulmonary eosinophil influx. The *in vivo* and *in vitro* anti-allergic effects of these compounds were not observed in AhR-knockout animals [49,53,54].



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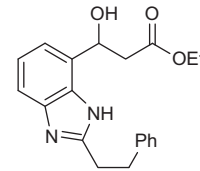

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4.4. Type 1 diabetes

The NOD mouse is a spontaneous model of human type 1 diabetes. As is believed to be true for the human disease, the NOD mouse model is characterized by pancreatic infiltration of autoreactive T cells. Effector T cell-mediated destruction of insulin-secreting beta cells leads to a large increase in blood glucose concentration. The standard of care is daily injection of exogenous insulin.

Treatment of female NOD mice, which harbor the low dioxin-affinity AhR^d allele, with dioxin prevented diabetes (as defined by blood glucose concentration) in all of the treated animals after 23 weeks, as compared to a >70% diabetes incidence in vehicle-treated animals [55]. This was accompanied by a 50% increase in the absolute and relative number of $CD4^+CD25^+Foxp3^+$ Tregs in pancreatic lymph nodes. This result is interesting in light of the substantial literature implicating dioxin exposure to insulin resistance and the risk of type 2 diabetes—a form of the disease where secreted insulin is insufficient to reduce blood glucose concentration—in humans [56].

4.5. Graft versus host disease

The rejection of allogenic transplanted tissue is the primary complication for transplant recipients. It is frequently described as a graft versus host disease, since it characterized by infiltration of host effector T cells into the allogenic tissue. The standard of care is treatment with immunosuppressants such as rapamycin, with attendant increased risk of infection.

In a mouse model of graft versus host disease, streptozotocin-induced diabetic Balb/c mice were transplanted with pancreatic islet allografts from nondiabetic C57/Bl6 mice. Transplanted mice pretreated with **15** had a 70% graft survival rate after 30 days, compared to a 0% rate for vehicle-treated animals. Harvesting of DCs from the allograft-surviving cohort 60 days after transplantation and transfer to a group of freshly transplanted mice afforded a similar 70% allograft survival rate after 30 days. Both the **15**

directly treated group and the DC adoptive transfer group had a higher frequency of splenic $CD4^+Foxp3^+$ Tregs as compared to vehicle- and naïve DC-treated animals [38].

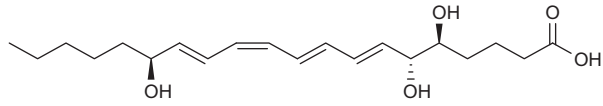
4.6. Experimental autoimmune uveitis (EAU)

Noninfectious uveitis is a frank inflammatory intraocular disease. The autoimmune form of the disease is distinguished by influx of autoreactive effector T cells into the posterior chamber of the eye and is frequently comorbid with an extraocular autoimmune disease, such as ankylosing spondylitis. If the affected tissue is not restored to its normally immune-privileged state, blindness typically results. The standard of care is treatment with steroids, frequently in combination with anti-rheumatoid arthritis drugs like methotrexate.

EAU can be induced in rodents by immunization with a retina-specific antigen, such as interphotoreceptor retinoid-binding protein. Subsequent ocular pathology includes retinal hemorrhage, retinal influx of $CD4^+$ effector T cells, and disruption of retinal cellular organization. In a mouse EAU model, a single 1 μg dose of dioxin given 1 day before disease induction completely inhibited development of disease after 21 days. Compared to vehicle-treated mice, dioxin-treated animals also had a 50% increase in splenocyte $CD4^+Foxp3^+$ Treg frequency, with a concomitant 90% reduction in antigen-stimulated IL-17A secretion *in vitro* [57].

4.7. Miscellaneous

The arachidonic acid metabolite lipoxin A_4 (**17**) has been characterized as a 300 nM potency AhR agonist in mouse liver cells [58]. In wild-type mice challenged with *T. gondii* soluble extract, lipoxin A_4 decreased spontaneous IL-12p40 secretion from splenocyte DCs by 75%, as compared to vehicle-treated animals. A 50% loss of lipoxin inhibitory efficacy was observed in AhR-knockout mice [59].



17

In wild-type mice, topical dosing to the ear of the previously mentioned SAhRM **9** inhibited phorbol ester-induced ear edema and mRNA induction for several inflammatory proteins, such as COX-2 and IL-6. These therapeutic effects were abrogated in AhR-knockout animals [27].

5. CONCLUSION

Extensive rodent studies reporting dioxin-induced, AhR-dependent wasting, immunosuppression, and carcinogenesis have suggested that AhR activation is a pathological event. However, recent evidence that dioxin at nontoxic doses (''the dose makes the poison'') [60] has therapeutic activity in rodent models of autoimmune diseases, with concomitant induction of Tregs and suppression of T_H17 development, has kindled interest in AhR agonism as a therapeutic target. This is supported by key findings that AhR activation-induced CYP protein induction is not inexorably toxic (as demonstrated in H/W rats); that several drug-like compounds (such as **13** and **14**) have AhR-dependent therapeutic activity in *in vivo* animal models of human disease; and that CYP induction can be decoupled from AhR-dependent therapeutic effects (as for **9**).

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