Resolving Inflammation by Targeting an Ancient Innate Immune Sensor with a Bacterial Metabolite

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Bacterial, fungal, and plant metabolites are promising sources of small molecules for drug discovery. Smith et al. show that tapinarof, a bacteria-derived polyphenol, is an aryl hydrocarbon receptor ligand that attenuated inflammatory responses and shows therapeutic potential for inflammatory skin diseases. This work adds to the growing literature stemming from the renewed interest in the discovery and development of novel small molecules with anti-inflammatory activity.

Smith et al. (2017) elucidate the mechanism of action of tapinarof, a bacteria-derived polyphenol that has therapeutic potential for both psoriasis and atopic dermatitis. Tapinarof (previously known as WBI-1001, GSK2894512), despite its striking structural similarity to the well-studied red wine polyphenol resveratrol (Figure 1a) (Park et al., 2012), has a different mechanism of action, targeting the aryl hydrocarbon receptor (AhR). This work adds to the growing literature stemming from the renewed interest in the discovery and development of small molecules for the treatment of inflammatory diseases.

Biotics targeting tumor necrosis factor, IL-17A, and related inflammatory cytokines (IL-1, IL-4/13R, IL-6, IL-12p40, IL-22, and IL-23p19), as well as other key molecules (CD20 and CTLA-4), have been developed and found to be highly effective for managing arthritis, psoriasis, and several other chronic inflammatory conditions. Despite their clinical efficacy, biologic drugs do have a number of notable drawbacks. Owing to their high molecular weight, biologic drugs must be administered by injection or infusion, and they are expensive to develop and manufacture. The use of biologics is also associated with adverse effects that can limit their use, and some biologics have been shown to lose efficacy over time (Gniadecki et al., 2015). Thus, despite the clinical successes of biologic drugs, there is still space in the physician’s armamentarium for small molecule drugs for the treatment of chronic inflammatory diseases.

Bacterial, fungal, and plant secondary metabolites are proving to be promising sources of biologically active small molecules for drug discovery. Medicinal plants have long been used as traditional remedies, and they are still used by over 75% of the world’s population to meet health care needs (World Health Organization, 2013). Traditional remedies remain a source of potential new therapeutics, with the identification of the bioactive phytochemicals aiding the development of new clinically useful drugs (Cragg and Newman, 2013). Plant extracts containing alkaloids (e.g., oleracrine), polyphenols (e.g., curcumin), flavonoids (e.g., quercetin and luteolin), glycosides (e.g., rhododendron), and acylphlorogluconols (e.g., rhodomyrtone) have all been investigated for their anti-inflammatory activities.

Screens are currently directed toward discovery of small molecules targeting intracellular pathways that are critical for inflammatory cytokine signal transduction. Some of these discoveries come in the wake of the successful targeting of tyrosine kinases in cancer therapy. In particular, molecules targeting the Janus kinase family (TYK2, JAK1, JAK2, and JAK3) are showing promise as inhibitors of intracellular signaling that is downstream of cytokine receptor engagement (Wesch et al., 2017). There are currently eight such inhibitors undergoing clinical trials for inflammatory skin diseases (clinicaltrials.gov), prompted by the success of tofacitinib, a small molecule JAK1/JAK3 inhibitor investigated both as an oral (rheumatoid arthritis, psoriasis, and alopecia) and topical agent (psoriasis, alopecia). These agents may be orally bioavailable or have topical effectiveness, the latter property permitting inhibition of inflammatory signaling at particular body sites without the global immune suppression seen with anti-cytokine biologics. An array of additional small molecule targets are currently being investigated, including RORC inhibitors, RIP kinase inhibitors, E2/E3 ubiquitin ligase inhibitors, and non-antibody IL-1 and IL-17A antagonists.

Evolutionarily, the AhR is highly conserved (Hahn et al., 2017) and appears to have developed in lockstep with our microbiota (Schiering et al., 2017). Early studies focused on AhR as a mediator of biological responses to xenobiotics or environmental contaminants such a halogenated aromatic hydrocarbons (e.g., 3,7,8-tetrachlorodibenzo-p-dioxin), diesel particulates, and tobacco smoke (benzo[α]pyrene). These notable environmental pollutants activate the AhR because of structural similarities with natural AhR ligands. More recently, the AhR has been recognized as a relevant participant in the innate immune system, acting as a sensor for tryptophan metabolites and a variety of polyaromatic hydrocarbons, including pigmented virulence factors from bacteria (Moura-Alveset al., 2014), with normal
physiological and homeostatic roles emerging for endogenous, food-derived (Stockinger et al., 2014), or microbe-derived AhR ligands (Schiering et al., 2017).

Functionally, AhR is a ligand-dependent transcription factor, a member of the basic helix-loop-helix-PAS protein family that includes the hypoxia sensor HIF1a/2a and the circadian protein CLOCK. These proteins require a binding partner, ARNT, for transcription factor activity (Stockinger et al., 2014). The AhR accumulates in the cytoplasm in an inactive form as a member of a protein complex with HSP90, AIP, and p23 (Figure 1b). Ligand binding induces conformational changes in the AhR, exposing its PAS-A domain and allowing AhR binding to ARNT. This shift in protein structure also exposes a nuclear localization sequence facilitating translocation of the complex to the nucleus. In the nucleus, ARNT progressively displaces HSP90 and the other cytoplasmic binding partners from AhR, increasing the affinity of the AhR-ARNT dimer for DNA. This dimeric transcription factor binds to xenobiotic response element (XRE) DNA sequences, driving the transcription of adjacent genes, notably several members of the cytochrome P450 superfamily of enzymes involved in metabolism of xenobiotics (CYP1A1, CYP1A2, and CYP1B1).

AhR is expressed by many types of cells, and its activity is controlled at several levels. First, as discussed, in the absence of ligand, AhR is held in an inactive state as part of a cytoplasmic complex. Second, by driving the expression of genes downstream of XRE sequences, AhR instigates a negative feedback loop that involves expression of the AhR repressor molecule, which disrupts nuclear AhR-ARNT complexes (Hahn et al., 2009). Finally, ligand-induced AhR activation leads to increased CYP1A1 expression, catalyzing the degradation of the AhR ligands themselves (Figure 1c). The exact outcome of AhR activation appears to depend on the cell types examined (because of differences in intracellular signaling and the availability of DNA-binding cofactors) and also the AhR ligands used, because opposing AhR activities have been seen using dioxin versus the endogenous ligand 6-formylindolo[3,2-b]carbazole (FICZ) (Ramirez et al., 2010). Further experimental differences in AhR ligand activities have been shown with local (topical) versus systemic administration of AhR ligands (Veldhoen et al., 2009).

AhR ligands have direct effects on a variety of immune cells, including dendritic cells, innate lymphoid cells, regulatory T cells, and T helper (Th) type 17 cells. However, functions of the AhR in stromal cells, such as the epithelia of the gut, lung, and skin, should not be overlooked, because it is these cells that form the primary interface with our environment and microbiota. AhR-deficient keratinocytes and fibroblasts have been shown to be hyper-responsive to inflammatory cytokines, and the AhR agonist FICZ can reduce skin inflammation in a mouse model, primarily via its action on keratinocytes and fibroblasts (Di Meglio et al., 2014). This anti-inflammatory consequence of AhR activation has been proposed as a mechanism that contributes to the efficacy of coal tar (Goeckerman) therapy for psoriasis. Coal tar contains a complex mixture of polyaromatic hydrocarbons, some of which trigger AhR, thereby reducing skin inflammation (van den Bogaard et al., 2013). Taken together, these observations are consistent with the notion that AhR expression by epithelia and resident immune cells may be one of the many ways we sense bacteria, and microbiota may tune our immune defenses at epithelial barriers via the AhR (Schiering et al., 2017).
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REFERENCES


Figure 1. Tapinarof suppresses inflammation via the aryl hydrocarbon receptor (AhR). (a) The bacterial metabolic tapinarof strongly resembles the structure of another well-studied natural product, resveratrol, but these molecules have contrasting mechanisms of action. (b) Tapinarof is a natural ligand for the AhR. AhR contains a basic helix-loop-helix domain for DNA binding, a PER-ARNT-SIM (PAS) domain containing PAS-A (ARNT binding) and PAS-B (ligand binding) repeat regions, and a glutamine (Q-rich) region involved in transcriptional activation. AhR is held in the cytoplasm as part of an inactive complex with HSP90, AIP, and p23. Upon ligand binding, conformational changes allow ARNT to bind to the complex to translocate to the nucleus. Once in the nucleus, HSP90, AIP, and p23 are shed and the AHR-ARNT dimer binds xenobiotic response element (XRE) DNA sequences to drive transcription of genes involved in cellular and immune function, which appears to be dependent on the availability of other DNA-binding co-factors and the nature of the ligand. As a negative feedback loop, AhR activation also induces the expression of a competitive inhibitor, AHRR, and several CYP450 enzymes that metabolize potential AhR ligands.