

A General Partnership between AhR and Tryptophan Catabolic Enzymes and Its Role in Endotoxin Tolerance

Marco Gargaro¹, Davide Matino¹, Matteo Pirro², Matteo Puccetti¹, Giulia Scalisi¹, Vincenzo Nicola Talesa¹, Ursula Grohmann¹, Antonio Macchiarulo³ and Francesca Fallarino^{1*}

¹Department of Experimental Medicine, University of Perugia, Perugia, Italy

²Department of Experimental Medicine, University of Perugia, Perugia, Italy

³Department of Chemistry and Technology of Drugs, University of Perugia, Italy

***Corresponding author:** Francesca Fallarino, Department of Experimental Medicine, University of Perugia, Perugia, Italy, Email: francesca.fallarino@unipg.it

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ABSTRACT

Tryptophan catabolites generated by tryptophan catabolic enzymes represent endogenous activators of the mammalian Aryl hydrocarbon Receptor (**AhR**), which is now credited with a variety of physiological functions. In both innate and adaptive immunity, one specific tryptophan catabolite, L-kynurenine, interacts with AhR to promote both genomic and nongenomic functions of AhR. Recently, we found that L-kynurenine and AhR activity are increased in a model of endotoxin tolerance. Here, we discuss the major factors that determine the outcome of gene transcriptional regulation by AhR. We focus on the impact of specific ligands in selecting different conformations of AhR, resulting in ligand-specific interactions within local tissue and cellular environments that will, in turn, provide specific sets of coactivators and functions, thus bridging the basic transcriptional machinery to the target genes. By interfacing with the generation and function of regulatory T cells in lymphoid tissues, tryptophan catabolites may be important physiological ligands of AhR in the regulation of immune responses as well as in sepsis.

Keywords: AhR; Kynurenine; IDO1; TDO2; LPS 2

MULTIPLE OUTCOMES OF AHR ACTIVATION

The Aryl hydrocarbon Receptor (**AhR**), a ligand-activated transcription factor, acts as a heterodimeric transcriptional regulator via association with the AhR Nuclear Translocator (**ARNT**). Widely expressed in a variety of animal species as well as in humans and initially credited with a specific function - namely, the hepatic clearance of xenobiotics, including dioxin - AhR has physiological functions that are only now beginning to be appreciated [1]. Much interest is now centered on the nature of its physiological ligands as well as on its mode of action in response to molecules that are different in nature as to their endogenous source and chemical structure [2]. Animal and human data indicate that AhR is involved in various signaling pathways critical to cell normal homeostasis and neoplasia as well [3], including cell proliferation and differentiation, gene regulation, cell motility and migration, inflammation and others [4,5].

Signal molecules function as ligands for AhR, and activated AhR binds promoter recognition sequences of the target genes. The AhR-ARNT complex may then require coactivators – including members of other families of transcription factors [6] - in order to initiate transcription and to unwind histone-bound DNA for exposing additional promoter recognition sites via their histone acetyltransferase function. Three major factors may affect the outcome of gene transcriptional regulation by AhR, namely, the nature of the ligand, the local tissue microenvironment, and the presence of coactivators in the cell. AhR activation by physiologically relevant ligands occurs in gut innate lymphoid cells by microbiota-derived Indole-3-Aldehyde (**IalD**) [7], in skin keratinocytes by endogenous 6-Formylindolo [3,2-B] Carbazole (**FICZ**) [8], and in lymphoid tissue dendritic cells by a product of tryptophan catabolic enzymes, L-kynurenine [9]. Here, we review the current understanding of the interplay between AhR and tryptophan-degrading enzymes in the regulation of immune responses, focusing on published data that have been providing significant new insight into the intricacies of AhR functioning.

TRYPTOPHAN CATABOLITES AS ENDOGENOUS AHR LIGANDS

The functions of AhR in T cells depend on the specific ligand bound to the receptor. For instance, binding of dioxin to AhR suppresses experimental autoimmune encephalomyelitis by promoting the development of Foxp3⁺ T regulatory (Treg) cells, whereas FICZ enhances encephalomyelitis by inducing the differentiation of IL-17-producing T cells [10]. Therefore, in determining the qualitative effect of AhR engagement, the ligand's ability to select a specific conformation of the receptor may be as important as its potency (determined by affinity) and efficacy. On considering the conformation-based operational model of agonism, it is likely that different AhR ligands preferentially bind distinct conformations of the AhR complex - each having a distinct set of fingerprint residues - thus initiating different pathways of downstream signaling and transcriptional events. Evidence in this direction comes from the finding that the AhR fingerprint residues required for activation by dioxin are distinct from those necessary for activation by L-kynurenine, even when the response being measured is the same, namely, transcription of a

gene (*Cyp1a1*) whose promoter contains xenobiotic response elements. A mutated form of the receptor that does not bind L-kynurenine will, instead, bind dioxin with increased potency and likely affinity [9]. This suggests that structurally and/or functionally distinct AhR ligands have distinct affinities for distinct conformations, each active conformation of the receptor recruiting a unique set of downstream signaling molecules (Figure 1). Thus, in a tissue, both ligand-intrinsic and cell-intrinsic factors dictate the outcome of AhR activation. The cell-intrinsic factors include competence for substrates that can be phosphorylated via a nongenomic function of AhR, namely, AhR complex-associated Src kinase activity. Of note, L-kynurenine promotes Src kinase activity on engaging AhR [9].

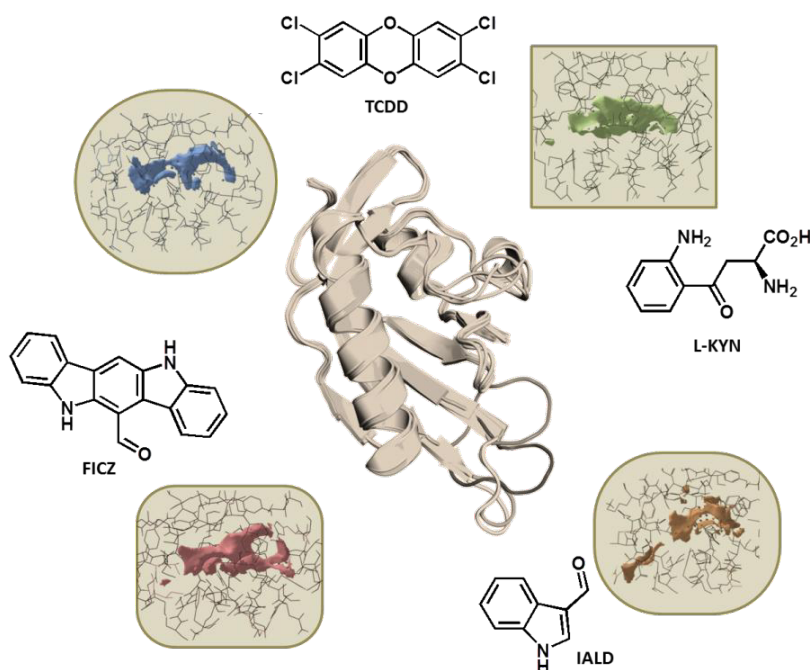


Figure 1: Binding mode of kynurenine (kyn) into the model of PAS-B AhR. Fingerprint residues involved in the binding of TCDD are shown in sticks and colored in green. Gln377 involved in hydrogen binding with the carboxylic group of kyn is shown in sticks and colored in yellow. Phe318 involved in cation- π interaction with the amino group of kyn is shown in sticks and colored in cyan.

Xenobiotics such as dietary components, products of microbiota, and ubiquitous environmental pollutants may have shaped this system in intestinal epithelia during millions of years of evolution. In the gut, a ligand for AhR can be derived or generated from diet, microbiome, and/or host cells. Mice on a tryptophan-enriched diet expand a population of lactobacilli in the gut that produce IALd, which promotes AhR-dependent transcription of the IL-22-encoding gene by host

innate lymphoid cells and thus preserves local homeostasis [7]. In the skin, AhR ligation controls oxidation/antioxidation, epidermal barrier function, photo-induced response, melanogenesis, and innate immunity. Dioxin-mediated skin and intestinal inflammation is associated with deregulated T-cell differentiation. In contrast, AhR activation by endogenous FICZ in keratinocytes mitigates the severity of skin inflammation [8].

In infection, AhR may sense distinct bacterial virulence factors and control antibacterial responses, thus contributing to infection resistance [11]. However, AhR contributes to “disease tolerance” as well [12,13]. In over inflammatory responses induced by infection, two distinct tryptophan catabolic enzymes, hepatic Tryptophan 2,3-dioxygenase (**TD02**) and ubiquitous Indoleamine 2,3-dioxygenase 1 (**IDO1**), produce L-kynurenine, that down modulates inflammatory cytokine gene transcription, and induces, instead, transcription of the genes encoding anti-inflammatory IL-10 and TGF- β . This mechanism involves the participation of noncanonical NF- κ B family members and other molecules including phosphorylated IDO1, which acts as a substrate for AhR complex-associated Src kinase activity [9]. All of these events are most clearly exemplified by the mechanisms of endotoxin tolerance, a condition whereby a prior exposure to a low level of LPS induces a durable state of cell refractoriness to subsequent LPS challenge [14-17], eventually leading to reprogramming of gene expression [18] and chromatin remodeling [19], and thus to a state of durable disease tolerance (Box 1). In this context, therefore, disease tolerance describes the ability of an infected host to limit disease severity without necessarily impacting the causative pathogen. This will preserve host reproductive capacity and survival (“fitness”) when eliminating the pathogen cannot be the prevailing strategy of host’s defense [20]. The issue of tissue damage control in disease tolerance has recently been reviewed with considerable detail [21].

Therefore, there is now strong evidence to support the concept that the outcome of AhR activation is largely dictated by the nature of the ligand - which initiates a specific sequence of downstream signaling events - as well as by the specific tissue (e.g., gut, skin, and lymphoid tissue) in which AhR engagement occurs.

AHR AND TRYPTOPHAN: MUTUALLY SUPPORTING FUNCTIONS

The above considerations allow further insight into the mutually supportive relationship between tryptophan catabolic enzymes and AhR in reinstalling homeostatic tolerance after meeting the needs of an acute inflammatory reaction, a condition under which tolerance is dangerously - yet inevitably - broken. This calls into play the pivotal roles of TD02 and IL-6 [9].

The combined effects of AhR and IDO1 act in feed forward loop, and yet they are self-regulated. AhR needs kynurenine to become activated and promote transcriptional activation of IDO1 [22], further relying on coactivators in order to give rise to a full range of gene transcription regulatory effects. Among those coactivators are members of the noncanonical NF- κ B family of transcription factors [23] and phosphorylated IDO1 itself, which - produced by AhR complex-associated Src kinase activity - acts as a signaling molecule and contributes to AhR-dependent gene regulation

[24]. AhR, which also acts as an E3 ubiquitin ligase [25], may then take full control over IDO1's dual - i.e., enzymic and signaling -function, by balancing Ido1 transcription with regulatory proteolysis of IDO1.

However, for activation of the AhR-IDO1 axis to occur, there must be no IL-6, which operates its own mode of IDO1 proteasomal degradation via induction of SOCS3, resulting in the enzyme ubiquitination and proteolysis [26]. This condition is clearly exemplified by chronic inflammatory conditions dominated by an overwhelming production of IL-6 [27]. Ligand-bound AhR may well repress Il6 transcription, either directly or via transcription of Il10, resorting to IL-10 as a means of opposing IL-6 production [23]. As long as the AhR-IDO1 axis is blocked by IL-6, the AhR ligand kynurenine must derive from another tryptophan catabolic source.

At the onset of an inflammatory response, IL-6 production is a necessary, defensive mechanism that need not be abrogated till the onset of the transcriptional transition that will contribute to reinstall an optimal balance between inflammation and tolerance. Among the early events of systemic inflammation is cortisol-driven induction of acute phase proteins in liver. TDO2 is one such protein, and it is synthesized when production of proinflammatory cytokines, including Il-6, is at its height. Tdo2 is not under AhR transcriptional control, nor is TDO2 subjected to IL-6-driven proteasomal degradation [9]. This makes TDO2 production of kynurenine - and AhR activation - compatible with the early events in an inflammatory response. Thus, the roles of TDO2 and IDO1 as a source of AhR ligands are not redundant, the major differences lying in the different modalities of their respective gene activation, their being temporally segregated in function, and their disparate susceptibilities to inhibition by IL-6 (Figure 2).

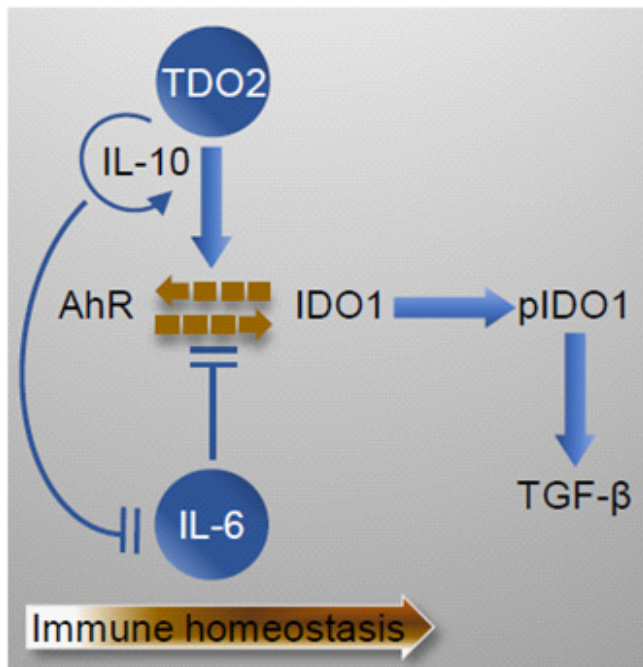


Figure 2: Nonredundant roles of TDO2 and IDO1 in inflammatory contexts. TDO2, not amenable to IL-6-driven proteasomal degradation, provides kynurenine as an activating ligand for AhR early in an inflammatory process. Accumulating noncanonical NF- κ B family members induce expression of IL-10, which opposes IL-6 production, thus releasing the AhR-IDO1 from IL-6 constraint.

AHR AND TRYPTOPHAN IN ENDOTOXIN TOLERANCE

The distinct contributions of TDO2 and IDO1 are clearly illustrated by the transition from Lipopolysaccharide (**LPS**) susceptibility to endotoxin tolerance, which exemplifies a mechanism of disease tolerance functionally similar to the acquisition of antigen-specific memory in acquired immunity. Endotoxin tolerance is, indeed, a mechanism whereby an initial exposure to LPS induces a durable state of resistance to subsequent endotoxemia [15,16,18,19]. A recent study in mice found that overreacting responses to primary LPS challenge are controlled by AhR, TDO2, and IL-10, whereas endotoxin tolerance requires the combined effects of AhR, IDO1, and TGF- β [9]. In particular, the IDO1-dependent, systemic regulatory response resulting in endotoxin tolerance appears to be part of a sequential series of events, to which AhR contributes distinct effects, mostly occurring in conventional dendritic cells. Intraperitoneal administration of an LPS sublethal dose promotes activation of hepatic TDO2 that produces kynurenine production despite the presence of high levels of circulating IL-6. Kynurenine, by acting as an AhR ligand increases IL-10 and decreases IL-1 β , TNF- α and IL-6 production, thus leading to reduced systemic inflammation. These events lead to the onset of endotoxin tolerance. High-dose LPS rechallenge in primed mice triggers AhR-Src kinase-dependent IDO1 phosphorylation, and TGF- β production

in splenic dendritic cells, and IDO1 expression by dendritic cells further increases kynurenine production. LPS-tolerant mice challenged with either gram-negative (*Salmonella enterica* Typhimurium) or gram-positive (group B streptococci) bacteria are less prone than unprimed controls to inflammatory pathology via a mechanism of disease tolerance (Figure 3) of interest, the signaling function of IDO1 is not restricted to conventional dendritic cells - where it occurs via Src activity - but may represent a generalized phenomenon in different types of immune response, with the involvement of several cell types, including plasmacytoid dendritic cells, in which the kinase Fyn is instead required [24].

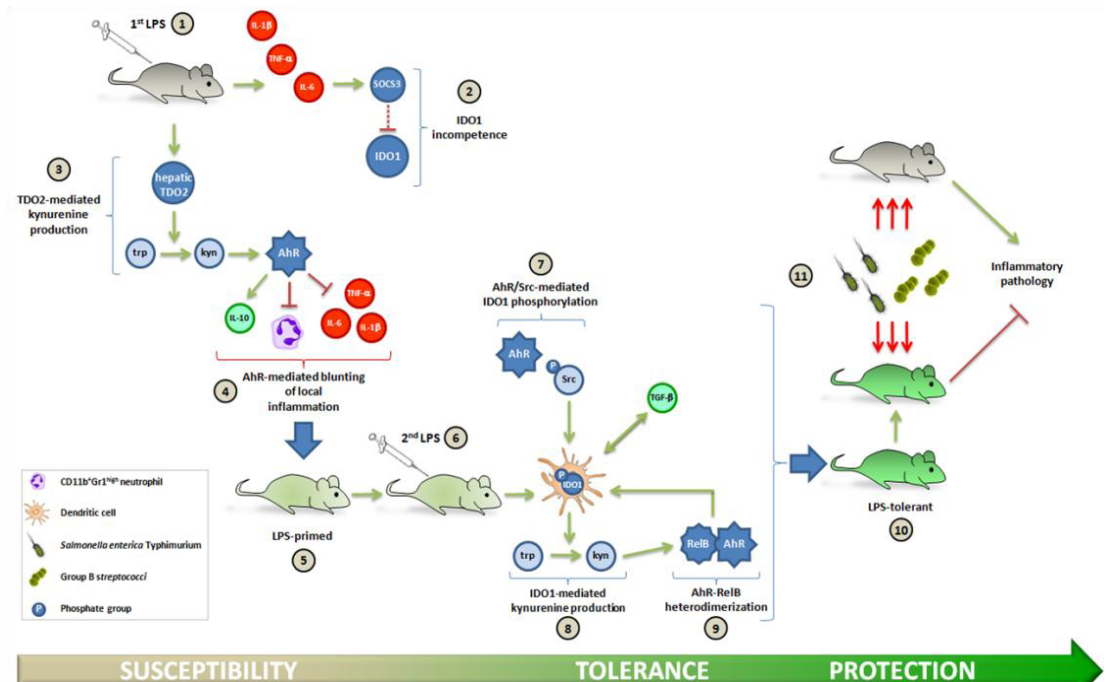


Figure 3: Schematic representation of the role of tryptophan catabolism in the transition from LPS susceptibility to tolerance leading to increased host fitness to immunopathology. (1) Intraperitoneal administration of an LPS sublethal dose (10 mg/kg) promotes activation of hepatic IDO2 that mediates the cleavage of the 2,3-double bond of the indole ring of tryptophan (trp) leading to kynurenine (kyn) production (2). (3) Kynurenine, by acting as an AhR ligand increases IL-10 and decreases IL-1 β , TNF- α and IL-6 production, thus leading to reduced systemic inflammation. (4) Events 1-3 lead to the onset of endotoxin tolerance. (5) High-dose LPS rechallenge (40 mg/kg) in primed mice triggers AhR-Src kinase-dependent IDO1 phosphorylation (6), and TGF- β production in splenic dendritic cells. (7) IDO1 expression by dendritic cells further increases kynurenine production. (8) Events 5-7 lead to a fully LPS-tolerant state. (9) LPS-tolerant mice challenged with either gram-negative (*Salmonella enterica* Typhimurium) or gram-positive (group B streptococci) bacteria are less prone than unprimed controls to inflammatory pathology (10).

THE COEVOLUTION OF IDO1, AHR, AND TREG CELLS IN ACQUIRED IMMUNITY

AhR is presumed to have evolved from invertebrates, where it served a ligand-independent role in normal development processes. Evolution of the receptor in vertebrates resulted in the ability to bind structurally different ligands, including xenobiotics and microbiota-derived catabolites. Considering the inability of invertebrate AhR homologues to bind dioxins, the adaptive role of the AhR to act as a regulator of xenobiotic-metabolizing enzymes may have been a vertebrate innovation [1,28]. IDO1, in turn, has been conserved through phylogenesis for at least 600 million years, initially acting as a biostatic mechanism in the competition between tryptophan auxotroph and nonauxotrophic microorganisms [29]. Essentially the same biostatic function was likely conserved later in evolution, when IDO1 and Interferons (**IFNs**) - strong transcriptional inducers of its expression - emerged as key players in host antiviral defense and in resistance to intracellular pathogens, by depleting the essential amino acid tryptophan [30]. It was probably then that the same IFN-IDO1 axis became engaged in preventing eventually harmful, exaggerated inflammatory responses in the host, inflammation being often a dangerous necessity for the host to cope with infectious challenges [7,31,32]. This interpretation is compatible with the recent finding that a low dosage of a TLR9 agonist induces a proinflammatory signaling pathway, whereas a higher dosage triggers an alternate cascade of signaling events, culminating in tolerogenic IDO1-dependent functions [33]. Accordingly, IDO1 can meet apparently disparate environmental needs in plasmacytoid dendritic cells. Locally produced cytokines can turn IDO1's functional mode from one characterized by an intense but short course of tryptophan consumption (e.g., in IFN- γ -dominated innate or inflammatory responses) to a condition whereby IDO1 mediates a TGF- β -driven, self-maintaining form of intracellular signaling activity, which - independently of tryptophan degradation - contributes to sustaining a stable regulatory phenotype in those cells, as required by homeostatic tolerance [24,34].

A prototypical form of homeostatic tolerance occurs in mammalian pregnancy, thus preventing a semiallogeneic fetus rejection. Both IDO1 [35] and Treg cells [36] are required for the onset and maintenance of this type of tolerance. Because kynurenine contributes via AhR to Treg cell generation [37-39] and function [40-42], and because those cells have emerged in mammals to specifically protect the fetus [43], it was probably at time that IDO1 and AhR crossed their pathways to further coevolve with Treg cells, so to become a pivotal means of generating tolerance in a variety of pathophysiologic conditions in humans [31,40,42,44,45].

CONCLUDING REMARKS

Evidence that the Ah locus encoded a receptor was drawn from toxicology studies of dioxin. Known endogenous AhR ligands represent chemicals that are synthesized in higher organisms. Indigoids, equilenin, arachidonic acid metabolites, heme metabolites and tryptophan metabolites are examples of endogenous AhR ligands [2]. However, there is much evidence to support the

notion that endogenous AhR ligands play important roles in mammals. Tryptophan catabolites generated by TDO2 and IDO1 represent endogenous activators of the mammalian AhR. The IDO1 mechanism appears to have been selected through phylogenesis primarily to prevent over-reacting responses to TLR-recognized pathogen-associated molecular patterns [46,47], and only later did it become involved in the response to T cell receptor-recognized antigens [37,40,41,45]. As a result, in mammals, IDO1 has become pivotal in fetomaternal tolerance [35], at a time when regulatory T cells emerged to meet the same need, namely protecting the fetus [43]. IDO1 and regulatory T cells may have then coevolved to broaden their function well beyond their initial task of protecting the fetus [34], such that, in acquired immunity, IDO1 - with its dual enzymic and signaling function - has turned into an important component of the peripheral generation [37,38,48] and effector function [40,41] of regulatory T cells. By interfacing with the generation and function of Treg cells, tryptophan catabolites and indole molecules may rank among the most important physiological ligands of AhR.

BOX 1: ENDOTOXIN TOLERANCE

Lipopolysaccharide (LPS, endotoxin) is a structural component of the gram-negative outer membrane. The lipid A moiety of LPS binds to the LPS receptor complex expressed by leukocytes, endothelial cells, and parenchymal cells and is the primary component of gram-negative bacteria that is recognized by the immune system. Activation of the LPS receptor complex by native lipid A induces robust cytokine production, leukocyte activation, and inflammation, which is beneficial for clearing bacterial infections at the local level but can cause severe systemic inflammation and shock at higher challenge doses. Interestingly, prior exposure to LPS renders the host resistant to shock caused by subsequent LPS challenge, a phenomenon known as LPS or endotoxin tolerance [49]. LPS tolerance is thus an essential immune homeostatic response to repeated exposure to LPS that prevents excessive inflammatory responses. LPS tolerance induces a state of altered responsiveness in a variety of cells, including dendritic cells and macrophages, resulting in repression of proinflammatory gene expression and increased expression of factors that mediate resolution of inflammation. Although LPS tolerance is a transient state and the expression of proinflammatory genes may, with time, be restored to levels comparable to the acute response to LPS, a number of genes remain locked in a tolerizable state, while other genes are uniquely induced following recovery from LPS tolerance. Thus LPS-responsive cells adopt a unique transcriptional profile on recovery from LPS tolerance and have a distinct expression pattern of regulators of antigen presentation and transcription factors [50].

Because endotoxin tolerance - not merely amounting to a shutdown of LPS-induced responses - involves reprogramming of gene expression [18] and chromatin remodeling [19] - there is a resurgence of interest in this effect. Pharmacologic modulation of LPS-responsive genes to accelerate the onset of endotoxin tolerance would be beneficial in clinical settings dominated by acute hyperinflammatory responses to infection [18]. Perhaps even more intriguing is the possibility of exploiting endotoxin tolerance as a therapeutic maneuver. Not only endotoxin derived from gram-negative bacteria has immunomodulatory and allergy-protective potential [51], but endotoxin tolerance could help treat infections and prevent infection-associated immunopathology. TLR4 stimulation before or after *Streptococcus pneumoniae* infection improves survival by mechanisms dependent on T-cells [52], and endotoxin tolerance is likewise protective against in a *Streptococcus*-induced multifocal septic arthritis model [9]. These considerations may pave the way to developing a class of TLR4 agonists that mimic at least part of endotoxin tolerance functionality, which may eventually turn out to be immunoadjuvant-like drugs [49].

References

1. Stockinger B, Di Meglio P, Gialitakis M, Duarte JH. The aryl hydrocarbon receptor: multitasking in the immune system. *Annu Rev Immunol*. 2014; 32: 403-432.
2. Nguyen LP, Bradfield CA. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol*. 2008; 21: 102-116.
3. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*. 2011; 478: 197-203.
4. Stejskalova L, Dvorak Z, Pavek P. Endogenous and exogenous ligands of aryl hydrocarbon receptor: current state of art. *Curr Drug Metab*. 2011; 12: 198-212.
5. Nguyen NT, Hanieh H, Nakahama T, Kishimoto T. The roles of aryl hydrocarbon receptor in immune responses. *International immunology*. 2013; 25: 335-343.
6. Vogel CF, Khan EM, Leung PS, Gershwin ME, Chang WL, et al. Cross-talk between aryl hydrocarbon receptor and the inflammatory response: a role for nuclear factor- κ B. *J Biol Chem*. 2014; 289: 1866-1875.
7. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 2013; 39: 372-385.
8. Di Meglio P, Duarte JH, Ahlfors H, Owens ND, Li Y, et al. Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity*. 2014; 40: 989-1001.
9. Bessede A, Gargaro M, Pallotta MT, Matino D, Servillo G, et al. Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature*. 2014; 511: 184-190.
10. Quintana FJ. The aryl hydrocarbon receptor: a molecular pathway for the environmental control of the immune response. *Immunology*. 2013; 138: 183-189.
11. Moura-Alves P, Faé K, Houthuys E, Dorhoi A, Kreuchwig A, et al. AhR sensing of bacterial pigments regulates antibacterial defence. *Nature*. 2014; 512: 387-392.
12. Quintana FJ. LeA(H)Rning self-control. *Cell Res*. 2014; 24: 1155-1156.
13. Kundu P, Pettersson S. Immunology: Mammalian watchdog targets bacteria. *Nature*. 2014; 512: 377-378.
14. Fan H, Cook JA. Molecular mechanisms of endotoxin tolerance. *J Endotoxin Res*. 2004; 10: 71-84.
15. Pena OM1, Pistolic J, Raj D, Fjell CD, Hancock RE. Endotoxin tolerance represents a distinctive state of alternative polarization (M2) in human mononuclear cells. *J Immunol*. 2011; 186: 7243-7254.
16. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol*. 2011; 12: 231-238.
17. Abdi K, Singh NJ, Matzinger P. Lipopolysaccharide-activated dendritic cells: "exhausted" or alert and waiting? *J Immunol*. 2012; 188: 5981-5989.
18. Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol*. 2009; 30: 475-487.
19. Park SH, Park-Min KH, Chen J, Hu X, Ivashkiv LB. Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. *Nat Immunol*. 2011; 12: 607-615.
20. Soares MP. "Nuts and bolts" of disease tolerance. *Immunity*. 2014; 41: 176-178.
21. Soares MP, Gozzelino R, Weis S. Tissue damage control in disease tolerance. *Trends Immunol*. 2014; 35: 483-494.
22. Vogel CF, Goth SR, Dong B, Pessah IN, Matsumura F. Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochem Biophys Res Commun*. 2008; 375: 331-335.
23. Lawrence T, Bebie M, Liu GY, Nizet V, Karin M. IKK α limits macrophage NF- κ B activation and contributes to the resolution of inflammation. *Nature*. 2005; 434: 1138-1143.
24. Pallotta MT, Orabona C, Volpi C, Vacca C, Belladonna ML, et al. Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. *Nat Immunol*. 2011; 12: 870-878.
25. Ohtake F, Fujii-Kuriyama Y, Kato S. AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions. *Biochem Pharmacol*. 2009; 77: 474-484.
26. Orabona C, Pallotta MT, Volpi C, Fallarino F, Vacca C, et al. SOCS3 drives proteasomal degradation of indoleamine 2,3-dioxygenase (IDO) and antagonizes IDO-dependent tolerogenesis. *Proc Natl Acad Sci U S A*. 2008; 105: 20828-20833.

27. Romani L, Fallarino F, De Luca A, Montagnoli C, D'Angelo C, Zelante T, Vacca C. Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. *Nature*. 2008; 451: 211-215.
28. Quintana FJ, Sherr DH. Aryl hydrocarbon receptor control of adaptive immunity. *Pharmacol Rev*. 2013; 65: 1148-1161.
29. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol*. 2003; 24: 242-248.
30. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004; 4: 762-774.
31. Puccetti P, Grohmann U. IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. *Nat Rev Immunol*. 2007; 7: 817-823.
32. Volpi C, Giada Mondanelli, Paolo Puccetti, Ursula Grohmann. TLRs and tryptophan metabolism at the crossroad of immunoregulatory pathways. *Immunometabolism*. 2014; 1, 28-50.
33. Volpi C, Fallarino F, Pallotta MT, Bianchi R, Vacca C, et al. High doses of CpG oligodeoxynucleotides stimulate a tolerogenic TLR9-TRIF pathway. *Nat Commun*. 2013; 4: 1852.
34. Fallarino F, Grohmann U, Puccetti P. Indoleamine 2,3-dioxygenase: from catalyst to signaling function. *Eur J Immunol*. 2012; 42: 1932-1937.
35. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*. 1998; 281: 1191-1193.
36. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol*. 2004; 5: 266-271.
37. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol*. 2006; 176: 6752-6761.
38. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, et al. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol*. 2010; 185: 3190-3198.
39. Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A*. 2010; 107: 19961-19966.
40. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol*. 2002; 3: 1097-1101.
41. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol*. 2003; 4: 1206-1212.
42. Grohmann U, Volpi C, Fallarino F, Bozza S, Bianchi R, et al. Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. *Nat Med*. 2007; 13: 579-586.
43. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell*. 2012; 150: 29-38.
44. Grohmann U, Fallarino F, Bianchi R, Orabona C, Vacca C, Fioretti MC, Puccetti P. A defect in tryptophan catabolism impairs tolerance in nonobese diabetic mice. *J Exp Med*. 2003; 198: 153-160.
45. Fallarino F, Volpi C, Zelante T, Vacca C, Calvitti M, et al. IDO mediates TLR9-driven protection from experimental autoimmune diabetes. *J Immunol*. 2009; 183: 6303-6312.
46. Wingender G, Garbi N, Schumak B, Jüngerkes F, Endl E, et al. Systemic application of CpG-rich DNA suppresses adaptive T cell immunity via induction of IDO. *Eur J Immunol*. 2006; 36: 12-20.
47. Zelante T, Fallarino F, Bistoni F, Puccetti P, Romani L. Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. *Microbes Infect*. 2009; 11: 133-141.
48. Sucher R, Fischler K, Oberhuber R, Kronberger I, Margreiter C, et al. IDO and regulatory T cell support are critical for cytotoxic T lymphocyte-associated Ag-4 Ig-mediated long-term solid organ allograft survival. *J Immunol*. 2012; 188: 37-46.
49. Bohannon JK, Hernandez A, Enkhbaatar P, Adams WL, Sherwood ER. The immunobiology of toll-like receptor 4 agonists: from endotoxin tolerance to immunoadjuvants. *Shock*. 2013; 40: 451-462.
50. O'Carroll C, Fagan A, Shanahan F, Carmody RJ. Identification of a unique hybrid macrophage-polarization state following recovery from lipopolysaccharide tolerance. *Journal of immunology*. 2014; 192, 427-436.
51. Doreswamy V, Peden DB. Modulation of asthma by endotoxin. *Clin Exp Allergy*. 2011; 41: 9-19.
52. Musie E, Moore CC, Martin EN, Scheld WM. Toll-like receptor 4 stimulation before or after *Streptococcus pneumoniae* induced sepsis improves survival and is dependent on T-cells. *PLoS One*. 2014; 9: e86015.