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TRYPTOPHAN METABOLITES AND ARYL HYDROCARBON RECEPTOR SIGNALLING BY GUT MICROBES

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SUMMARY

Fungal diseases represent an important paradigm in immunology since they can result from either the lack of recognition or over-activation of the inflammatory response. Current understanding of the pathophysiology underlying fungal infections and diseases highlights the multiple cell populations and cell-signalling pathways involved in these complex conditions. A systems biology approach that integrates investigations of immunity at the systems-level is required to generate novel insights into this complexity and to decipher the dynamics of the host-fungus interaction. It is now clear that a three-way interaction between host, fungi, and microbiota dictates the types of host-fungus relationship. Metagenomics has revealed the complex interactions between fungal and bacterial commensals that, either directly or through the participation of the host immune system, impact on immune homeostasis at mucosal surfaces that, in turn, lead to secondary fungal infections. Metabolomics has captured the dialogue between the mammalian host and its microbiota. The host tryptophan catabolic enzyme, indoleamine 2,3-dioxygenase 1 (IDO1) plays a dominant role in the interplay between tryptophan catabolism by microbial communities, the host's own pathway of metabolite production, and the activation of the aryl hydrocarbon receptor (AhR)/IL-22 axis, eventually impacting on mucosal immune homeostasis and host/fungal symbiosis. Thus, the regulatory loop involving AhR and IDO1 may be exploited for the development of multi-pronged-host- and microbiota-directed therapeutic approaches for mucosal and systemic fungal diseases.

FUNGI ENTER THE METAGENOMIC ERA

Fungi can interact with their hosts (plants, animals or humans) in multiple ways, establishing symbiotic, commensal or pathogenic relationships. Most fungi, such as *Aspergillus fumigatus*, *Cryptococcus neoformans*, and the thermally dimorphic fungi (*Histoplasma capsulatum, Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Penicillium marneffei*, and *Sporothrix schenckii*), are ubiquitous in the environment and humans are exposed by inhaling spores or small yeast cells. As a result, they can interact with humans in multiple ways, establishing symbiotic, commensal, latent or pathogenic relationships

However, among eukaryotes, fungi are particularly prominent residents of the human body (*Huffnagle* and *Noverr*, 2013). More than 400 species of fungi associated with human beings

have been identified (*Cui,* et al., 2013). Very little is known about the biology of the members of the human "mycobiome" (i.e., fungal microbiome) and even less about the interactions that they establish with the human host. Thus, an increased understanding of the importance of mycobiota in shaping the host's immune and metabolic activities will render fungal interactions with their hosts more complex than previously appreciated (*Romani,* et al., 2014). As a corollary, early diagnosis may no longer be a challenge for invasive or chronic fungal diseases, as suggested (*Schelenz,* et al., 2015). Instead, the study of the human mycobiota in the trans-omics era, with a focus on metagenomics and metabonomics, is providing novel insights into the regulation of host/fungus immune homeostasis. Evidence is accumulating to support the exciting concept that the interaction between different biomes and between the host and the mycobiome are critical in the pathogenesis of fungal infections and other human diseases (*Cui,* et al., 2013; *Huffnagle* and *Noverr*, 2013; *Seed*, 2014; *Underhill* and *Iliev*, 2014).

THE HUMAN MYCOBIOME

The development of culture-independent methods has expanded our knowledge of the mycobiomes found in different body sites, their interface with other biomes and their association with human health and diseases (*Cui* et al., 2013). Alterations in the mycobiome are frequently reported to be associated with various diseases such as cystic fibrosis (CF) (*Delhaes* et al., 2012), inflammatory bowel diseases (*Ott* et al., 2008; *Li* et al., 2014;), atopic dermatitis (*Zhang* et al., 2011) or mucocutaneous candidiasis (*Smeekens* et al., 2014). However, it remains to be elucidated whether this variation is primary or secondary to an imbalanced bacterial microbiome. Indeed, interactions of fungi with bacteria *in vitro* have been described (reviewed in: *Wang* et al., 2014) as well as the clinical relevance of these interactions (*Peleg* et al., 2010), such as the occurrence of intractable candidiasis in association with antibiotic-induced dysbiosis (*Krause* et al., 2001) and of mixed fungal-bacterial species in biofilms (*Peleg* et al., 2010). Fungal-bacterial interactions can be antagonistic, synergistic or symbiotic; regardless they influence the physiological characteristics and survival of either one partner and, consequently, impact on host immune reactivity. Variations in the mycobiome can also be secondary to dysregulated host immune reactivity. The traditional view of a single direction by which bacteria stimulate the immune system, leading to inflammation or autoimmune disorders, has been challenged by a more complex view: the gut immune system does not simply protect from pathogens, but is actively involved in the maintenance of a rich and healthy community of gut bacteria (*Kawamoto* et al., 2014). Faults in the immune regulation lead to changes in the bacterial community that in turn feed back into the immune system. Similar to the microbiome, the host/mycobiome interactions also lead to mutual influences. Not only is the host affecting the mycobiome composition and variations by means of genotype, physiology, immune system, and lifestyle but the fungal microbiota may contribute to the balance of inflammation and tolerance at local mucosal surfaces and at distal sites (*Noverr* and *Huffnagle*, 2004).

RESISTANCE AND TOLERANCE MECHANISMS OF ANTIFUNGAL IMMUNITY

As the immune system has evolved to accommodate colonization by symbiotic microbes while retaining the capacity to oppose their infectivity, a fine balance between pro- and anti-inflammatory signals is a prerequisite for a stable host/fungal relationship, the disruption of which may lead to pathological consequences. Indeed, despite the occurrence of severe fungal infections in immunocompromised patients, clinical evidence indicates that fungal diseases also occur in the setting of a heightened inflammatory response, in which immunity occurs at the expense of host damage and pathogen eradication (*Perfect*, 2012). A number of fungal diseases are critical examples of such bidirectional influences between infection and immune-related pathology, a condition that highlights the bipolar nature of the inflammatory process in infection. Early inflammation prevents or limits infection, but an uncontrolled response may eventually oppose disease eradication. This conceptual principle is best exemplified by the occurrence of severe fungal infections in patients with chronic granulomatous disease (*Romani* et al., 2008a), CF (*Iannitti* et al. 2013) or with immune reconstitution inflammatory syndrome (*Singh* and *Perfect*, 2007), an entity characterized by local and systemic inflammatory reactions that can result in quiescent or latent infections manifesting as opportunistic mycoses. Chronic mucocutaneous candidiasis (CMC) and chronic disseminated candidiasis also belongs to the spectrum of fungus-related immune reconstitution inflammatory syndrome (*Legrand* et al., 2008). Thus, an immune response that limits both fungal infectivity and host collateral damage is required to maintain a homeostatic environment (*Casadevall* and *Pirofski*, 2003). This dual role has recently been accommodated within the conceptual framework of a two-component antifungal immune response, i.e., resistance (the ability to limit fungal burden) and tolerance (the ability to limit the host damage caused by either the immune response or other mechanisms). Resistance is meant to reduce pathogen burden through innate and adaptive immune mechanisms whereas a plethora of tolerance mechanisms, despite less known relative to resistance mechanisms, protect the host from immune- or pathogen-induced damage (*Saraiva* and *O'Garra*, 2010).

MICROBIOTA REGULATION OF RESISTANCE AND TOLERANCE TO FUNGI VIA TRYPTOPHAN METABOLISM

The enzyme indoleamine 2,3-dioxygenase 1 (IDO1) and its downstream catabolites sustain the delicate balance between Th1/Th17 pathways and Treg cells, by providing the host with adequate protective immune mechanisms without necessarily eliminating the pathogen or causing undesirable tissue damage (*Zelante* et al., 2009). As a result of their ability to induce differentiation of Treg cells and inhibit Th17 cells, IDO1 is critical to cell lineage commitment in experimental fungal infections and contributes to the overall outcome of inflammation, allergy and Th17-driven inflammation in these infections. Under these circumstances, the Th17 pathway, by inhibiting tryptophan catabolism, may instead favour pathology and provides evidence

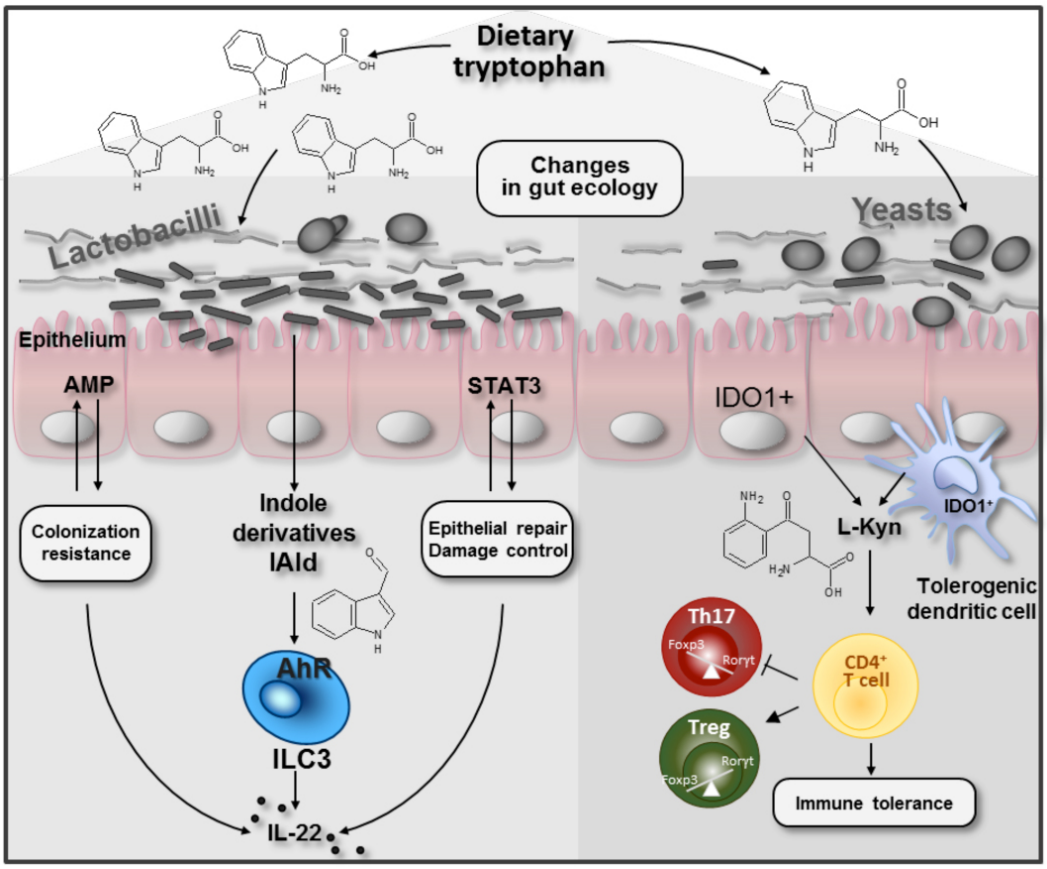

Figure 1: Resistance and tolerance to fungi and its regulation by tryptophan.
The tryptophan metabolism pathway is exploited by the mammalian host and commensals (including fungi) to increase fitness in response to fungi through resistance and tolerance. At mucosal surfaces and skin, the fungal biota promotes the production IL-22, via IL-23 and aryl hydrocarbon receptor (AhR) ligands, by $CD3^{-}NKp46^{+}$retinoic acid-related orphan receptor-γt $(ROR\gamma t)^{+}AhR^{+}$ innate lymphoid cells. By contrast, $NKp46^{-}$ cells produce IL-17A. IL-22 targets epithelial cells, leading to activation of signal transducer and activator of transcription 3 (STAT3) and, together with IL-17A, to production of antimicrobial peptides. Various indole derivatives, which are generated through conversion from dietary tryptophan by commensal intestinal microorganisms, act as endogenous ligands for AhR, and thereby contribute to IL-22 production. Fungus-induced activation of tryptophan catabolism by indoleamine 2,3–dioxygenase (IDO1) expressed by dendritic cells and epithelial cells leads to the production of immunologically active compounds that induce the transcription of FOXP3 and suppress the transcription of RORγt. These findings support a model in which the AhR/IL-22/IL-17A axis control initial fungal growth (i.e., resistance) and epithelial cell homeostasis. By contrast, the exploitation of the IFN-γ/IDO1 axis for functional specialization of antifungal regulatory mechanisms (i.e. tolerance) may have allowed the fungal microbiota to evolve with the mammalian immune system, survive in conditions of inflammation and prevent dysregulated immunity. The balance between resistance and tolerance to fungi may accommodate the spectrum of host/fungus relationships, ranging from protection and immunopathology to fungal persistence and immunosuppression.

accommodating the apparently paradoxical association of chronic inflammation with fungal disease (*Romani* et al., 2008a). IDO1 is a 'metabolic' enzyme conserved through the past 600 million years of evolution. Initially

recognized in infection because of antimicrobial activity ('tryptophan starvation' of intracellular parasites), IDO1 is now widely recognized as suppressor of acute inflammatory responses and regulator of mammalian immune homeostasis (*Zelante* et al., 2009). Not surprising, IDO1 may represent an evasion mechanism for microbes that establish commensalism or chronic infection (*Zelante* et al., 2009). In their capacity to induce Tregs and inhibit Th17, IDO1-expressing DCs and epithelial cells and kynurenines revealed an unexpected potential in the control of inflammation, allergy and Th17-driven inflammation in these infections (*Grohmann* et al., 2007; *Romani* et al., 2008b).

Commensal-driven mucosal responses are up-regulated in IDO1 deficiency (*Harrington* et al., 2008) and IL-22 responses are up-regulated in conditions of defective adaptive immunity (*De Luca* et al., 2010) and IDO deficiency (*Zelante* et al., 2013). AhR is a ligand-activated transcription factor that mediates IL-22 production (*Trifari* et al., 2009). A variety of indole derivatives act as endogenous ligands for AhR (*Heath-Pagliuso* et al., 1998) and are generated through conversion from dietary tryptophan by commensal intestinal microbes (*Bjeldanes* et al., 1991). Recent evidence has shown that AhR is involved in the (patho)physiology of skin including the regulation of skin pigmentation, photocarcinogenesis, and skin inflammation (*Esser* et al., 2013; *Di Meglio* et al., 2014). Of interest is the ability of *Malassezia*-derived indoles to activate AhR correlated with local immunoregulation (*Vlachos* et al., 2012) and pathogenicity in seborrhoeic dermatitis (*Gaitanis* et al., 2008). Similarly, metabolomics has revealed that bioactive indoles with AhR agonist activity are also present in mice with candidiasis (*Zelante* et al., 2013). Thus, the trpyptophan metabolism pathway is exploited by commensals and the mammalian host to increase fitness in response to fungi via induction of resistance and tolerance at the skin and mucosal surface. The new findings support a mode (Figure 1) in which the IL-22 axis controls the initial fungal growth (i.e., resistance) and epithelial cells homeostasis likely exploiting primitive anti-fungal effector defence mechanisms. In contrast, the exploitation of the IFN-γ/IDO1 axis for functional specialization of antifungal regulatory mechanisms (i.e. protective tolerance) may have allowed the fungal microbiota to co-evolute with the mammalian immune system, to survive in conditions of high-threat inflammation and to prevent dysregulated immunity (*Zelante* et al., 2009). The two pathways, although non-redundant, are reciprocally regulated and compensate each other in the relative absence of either one (*De Luca* et al., 2010), consistent with the theme that adaptive immunity depends on innate immunity but innate immunity requires adaptive regulation. This finding not only helps to explain the association of fungal infections with dysbiosis but also points to the essential help the microbiota may provide in fungal colonization and pathogenicity in immunodeficient patients.

ADVANCING HOST- AND MICROBIOTA-DIRECTED THERAPY FOR FUNGAL DISEASES ALONG THE AHR/IDO1 PATHWAY

A plethora of preclinical models suggests that the tryptophan to kynurenine immune tolerance pathway is active in cancer immunity, autoimmunity, infection, transplant rejection, and allergy. Drugs targeting this pathway are

already in clinical trials with the aim at reverting cancer-induced immunosuppression (*Platten* et al., 2014). However, the tryptophan to kynurenine pathway via IDO1 also plays a dominant role in the interplay between tryptophan catabolism by microbial communities, the host's own pathway of metabolite production, and the orchestration of AhR-dependent T-cell immune homeostasis. As AhR stimulation may lead in turn to IDO1 activation via an autocrine AhR-IL6-STAT3 signalling loop (*Litzenburger* et al., 2014), such a positive feed-forward loop between IDO1 and AhR may have allowed the mycobiota to co-evolve with the mammalian immune system, to survive under conditions of high-threat inflammation and to prevent dysregulated immunity in response to environmental cues. This implicates that the AhR/IDO1 loop could be exploited for rational host- and microbial-directed therapies in high-risk patients and suggests that antifungal therapy should consider inter-individual variations in the active human microbiome. Thus, challenging existing paradigms with new perspectives from the cross-talk between fungi, the immune system and the microbiota will eventually lead toward the development of multi-pronged therapeutic approaches for mucosal and systemic fungal diseases.

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LITERATURE

Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C. and Bradfield, C.A.: Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc. Natl. Acad. Sci. USA 88, 9543-9547 (1991).

Casadevall, A. and Pirofski, L.A.: The damage-response framework of microbial pathogenesis. Nat. Rev. Microbiol. 1, 17-24 (2003).

Cui, L., Morris, A. and Ghedin, E.: The human mycobiome in health and disease. Genome Med. 5, 63 (2013).

De Luca, A., Zelante, T., D'Angelo, C., Zagarella, S., Fallarino, F., Spreca, A., Iannitti, R.G., Bonifazi, P., Renauld, J.C., Bistoni, F., Puccetti, P. and Romani, L.: IL-22 defines a novel immune pathway of antifungal resistance. Mucosal Immunol. 3, 361-373 (2010).

Di Meglio, P., Duarte, J.H., Ahlfors, H., Owens, N.D., Li, Y., Villanova, F., Tosi, I., Hirota, K., Nestle, F.O., Mrowietz, U., Gilchrist, M.J. and Stockinger, B.: Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. Immunity 40, 989-1001 (2014).

Esser, C., Bargen, I., Weighardt, H., Haarmann-Stemmann, T. and Krutmann, J.: Functions of the aryl hydrocarbon receptor in the skin. Semin. Immunopathol. 35, 677-691 (2013).

Gaitanis, G., Magiatis, P., Stathopoulou, K., Bassukas, I.D., Alexopoulos, E.C., Velegraki, A. and Skaltsounis, A.L.: AhR ligands, malassezin, and indole[3,2-b] carbazole are selectively produced by Malassezia furfur strains isolated from

seborrheic dermatitis. J. Invest. Dermatol. 128, 1620-1625 (2008).

Grohmann, U., Volpi, C., Fallarino, F., Bozza, S., Bianchi, R., Vacca, C., Orabona, C., Belladonna, M.L., Ayroldi, E., Nocentini, G., Boon L., Bistoni, F., Fioretti, M.C., Romani, L., Riccardi, C. and Puccetti, P.: Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. Nat. Med. 13, 579-586 (2007).

Harrington, L., Srikanth, C.V., Antony, R., Rhee, S.J., Mellor, A.L., Shi, H.N. and Cherayil, B.J.: Deficiency of indoleamine 2,3-dioxygenase enhances commensal-induced antibody responses and protects against Citrobacter rodentium-induced colitis. Infect. Immun. 76, 3045-3053 (2008).

Heath-Pagliuso, S., Rogers, W.J., Tullis, K., Seidel, S.D., Cenijn, P.H., Brouwer, A. and Denison, M.S.: Activation of the Ah receptor by tryptophan and tryptophan metabolites. Biochemistry 37, 11508-11515 (1998).

Huffnagle, G.B. and Noverr, M.C.: The emerging world of the fungal microbiome. Trends Microbiol. 21, 334-341 (2013).

Iannitti, R.G., Carvalho, A., Cunha, C., De Luca, A., Giovannini, G., Casagrande, A., Zelante, T., Vacca, C., Fallarino, F., Puccetti, P., Massi-Benedetti, C., Defilippi, G., Russo, M., Porcaro, L., Colombo, C., Ratclif, L., De Benedictis, F.M. and Romani, L.: Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. Am. J. Respir. Crit. Care Med. 187, 609-620 (2013).

Legrand, F., Lecuit, M., Dupont, B., Bellaton, E., Huerre, M., Rohrlich, P.S. and Lortholary, O.: Adjuvant corticosteroid therapy for chronic disseminated candidiasis. Clin. Infect. Dis. 46, 696-702 (2008).

Litzenburger, U.M., Opitz, C.A., Sahm, F., Rauschenbach, K.J., Trump, S., Winter, M., Ott, M., Ochs, K., Lutz, C., Liu, X., Anastasov, N., Lehmann, I., Hofer, T., von Deimling, A., Wick, W. and Platten, M.: Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. Oncotarget 5, 1038-1051 (2014).

Perfect, J.R. The impact of the host on fungal infections. Am. J. Med. 125, S39-S51 (2012).

Platten, M., von Knebel Doeberitz, N., Oezen, I., Wick, W. and Ochs, K.: Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. Front. Immunol. 5, 673 (2014).

Romani, L., Fallarino, F., De Luca, A., Montagnoli, C., D'Angelo, C., Zelante, T., Vacca, C., Bistoni, F., Fioretti, M.C., Grohmann, U., Segal, B.H. and Puccetti, P.: Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. Nature 451, 211-215 (2008a).

Romani, L., Zelante, T., De Luca, A., Fallarino, F. and Puccetti, P.: IL-17 and therapeutic kynurenines in pathogenic inflammation to fungi. J. Immunol. 180, 5157-5162 (2008b).

Romani, L., Zelante, T., De Luca, A., Iannitti, R.G., Moretti, S., Bartoli, A., Aversa, F. and Puccetti, P.: Microbiota control of a tryptophan-AhR pathway in disease tolerance to fungi. Eur. J. Immunol. 44, 3192-3200 (2014).

Saraiva, M. and O'Garra, A.: The regulation of IL-10 production by immune cells. Nat. Rev. Immunol. 10, 170-181 (2010).

Schelenz, S., Barnes, R.A., Barton, R.C., Cleverley, J.R., Lucas, S.B., Kibbler, C.C., Denning, D.W. and British Society for Medical Mycology. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. Lancet Infect. Dis. 15, 461-474 (2015).

Seed, P.C.: The Human Mycobiome. Cold Spring Harb. Perspect. Med. 5, a019810 (2014).

Singh, N. and Perfect, J.R.: Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect. Dis. 7, 395-401 (2007).

Trifari, S., Kaplan, C.D., Tran, E.H., Crellin, N.K. and Spits, H.: Identification of a human helper T cell population that has

abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. Nat. Immunol. 10, 864-871 (2009).

Underhill, D.M. and Iliev, I.D.: The mycobiota: interactions between commensal fungi and the host immune system. Nat. Rev. Immunol. 14, 405-416 (2014).

Vlachos, C., Schulte, B.M., Magiatis, P., Adema, G.J. and Gaitanis, G.: Malassezia-derived indoles activate the aryl hydrocarbon receptor and inhibit Toll-like receptor-induced maturation in monocyte-derived dendritic cells. Br. J. Dermatol. 167, 496-505 (2012).

Zelante, T., Fallarino, F., Bistoni, F., Puccetti, P. and Romani, L.: Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. Microbes Infect. 11, 133-141 (2009).

Zelante, T., Iannitti, R.G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., Zecchi, R., D'Angelo, C., Massi-Benedetti, C., Fallarino, F., Carvalho, A., Puccetti, P. and Romani, L.: Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via Interleukin-22. Immunity 39, 372-385 (2013).