

# Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease

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The gut microbiota is a crucial actor in human physiology. Many of these effects are mediated by metabolites that are either produced by the microbes or derived from the transformation of environmental or host molecules. Among the array of metabolites at the interface between these microorganisms and the host is the essential aromatic amino acid tryptophan (Trp). In the gut, the three major Trp metabolism pathways leading to serotonin (5-hydroxytryptamine), kynurenine (Kyn), and indole derivatives are under the direct or indirect control of the microbiota. In this review, we gather the most recent advances concerning the central role of Trp metabolism in microbiota-host crosstalk in health and disease. Deciphering the complex equilibrium between these pathways will facilitate a better understanding of the pathogenesis of human diseases and open therapeutic opportunities.

## Introduction

The intestine is a complex ecosystem harboring a dense and diverse microbial community called the gut microbiota, which co-evolved with the host to develop a mutualistic relationship. It is increasingly obvious that loss of the fragile equilibrium within this complex ecosystem, termed dysbiosis, is implicated in numerous human diseases. The intestinal microbiota has an important impact on several key physiological host functions, including metabolic and nutritional homeostasis, immune system maturation and stimulation, and even brain activity. These effects are mediated by direct cell-to-cell interactions and by metabolites that are either produced by the microbes or derived from the transformation of environmental or host molecules. The gut microbiota is considered a virtual endocrine organ, producing molecules that are able to interact with the host physiology and trigger responses at the local and distant levels (Zhang and Davies, 2016). Any perturbation in host-microbiota crosstalk can be an initiating or reinforcing factor in disease pathogenesis. A large array of metabolites drives the crosstalk between the host and its microbiome. The three currently most studied categories of metabolites involved in host-microbiota interactions are (1) short-chain fatty acids (SCFAs), produced by bacteria from the fermentation of fibers; (2) bile acids produced in the liver and transformed by the gut microbiota before re-affecting the host; and (3) tryptophan (Trp) metabolites, which are the topic of this review (Blacher et al., 2017).

In this article, we review the most recent insights regarding the role of the gut microbiota in Trp metabolism, with a focus on the consequences on both physiology and diseases. In addition to the direct transformation of Trp into bioactive molecules by the gut microbiota, we discuss the way in which the gut microbiota controls host Trp metabolism in the gut.

## Tryptophan: Origin and Production

Trp is an essential aromatic amino acid composed of a  $\beta$  carbon connected to the 3 position of an indole group. Of the 20 com-

mon canonical amino acids, Trp is the largest by molecular weight. Although Trp is the least abundant amino acid in protein and cells, it is a biosynthetic precursor of a large number of microbial (Alkhalaf and Ryan, 2015) and host metabolites.

The ability of specific bacteria to produce Trp has been known for a century and has been significantly exploited in industry. Although some members of the bacterial microbiota, such as *Escherichia coli*, are able to produce Trp, no data support the significant contribution of bacteria-derived Trp in human physiology. Since Trp is not produced by animal cells, humans rely on exogenous, mostly dietary, intake. Common natural food sources of Trp include oats, bananas, dried prunes, milk, tuna fish, cheese, bread, poultry, peanuts, and chocolate. The World Health Organization set the recommended Trp intake to 4 mg/kg/day, and, to date, no adverse effects of excess Trp in the diet have been reported.

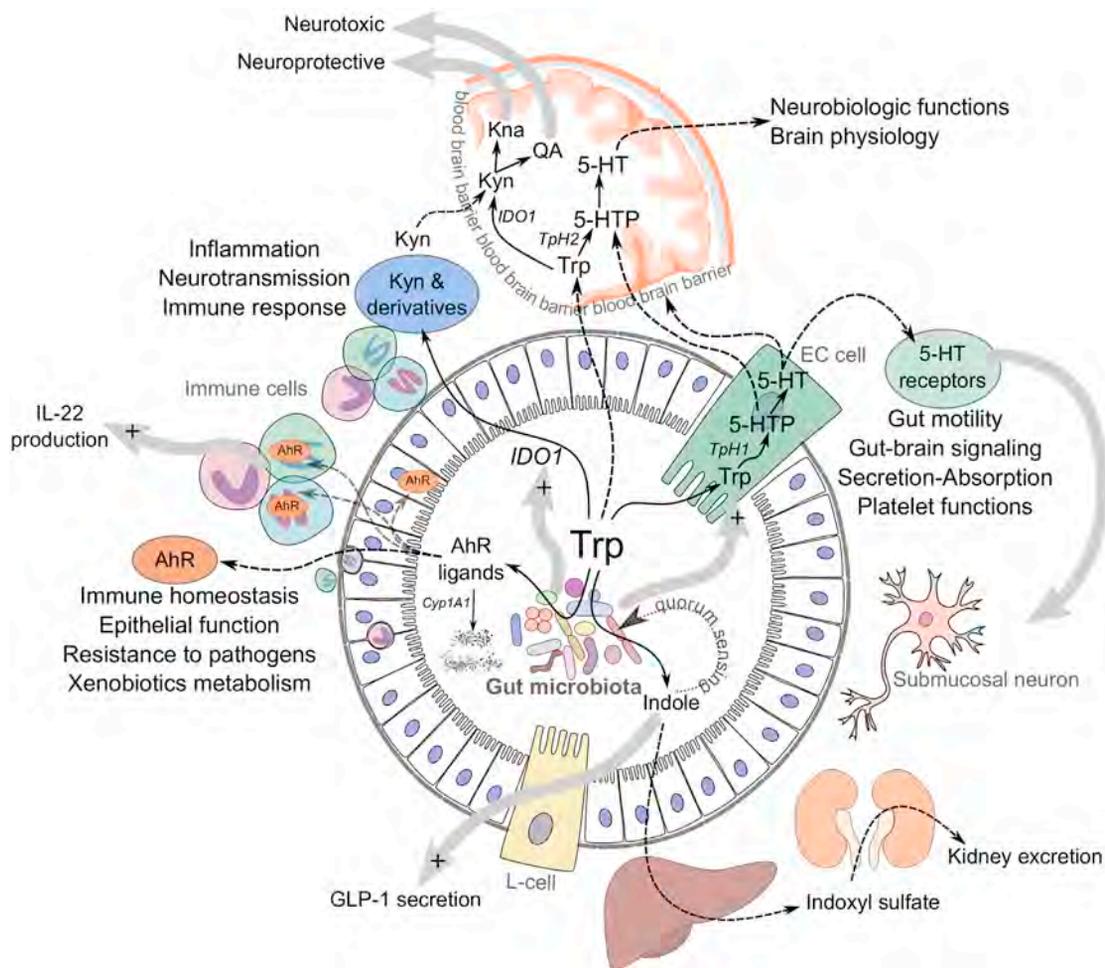
## Intestinal Tryptophan Metabolism: Pathways and Physiology

Trp metabolism follows three major pathways in the gastrointestinal tract (Figure 1): (1) the direct transformation of Trp into several molecules, including ligands of the aryl hydrocarbon receptor (AhR), by the gut microbiota (Zelante et al., 2013); (2) the kynurenine pathway (KP) in both immune and epithelial cells via indoleamine 2,3-dioxygenase (IDO) 1 (Clarke et al., 2013); and (3) the serotonin (5-hydroxytryptamine [5-HT]) production pathway in enterochromaffin cells via Trp hydroxylase 1 (Tph1) (Yano et al., 2015).

## Direct Metabolism of Trp by Microorganisms

Trp metabolism in the gut includes the direct transformation of Trp by intestinal microorganisms into several molecules, such as indole and its derivatives. Many indole derivatives, such as indole-3-aldehyde (IAld), indole-3-acid-acetic (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld), and indoleacrylic acid, are ligands for AhR (Alexeev et al., 2018;





**Figure 1. Integrated Trp Metabolism under the Control of the Gut Microbiota in Host Physiology**

Dietary Trp can be directly converted by gut microbiota into AhR ligands that are able to tune local and distant host functions, including immune homeostasis and barrier physiology. Gut microbiota also influence the kynurenine-producing IDO pathway, which plays a critical role in inflammatory mechanisms, immune responses, and neurobiological functions. Peripheral production of serotonin by enterochromaffin cells is also under the influence of the gut microbiota. Gut-produced serotonin has many local effects, such as stimulating gut motility, and, even if it does not cross the blood-brain barrier, gut microbiota indirectly affects central serotonergic pathways by modulating Trp and tryptamine availability. 5-HTP, 5-hydroxytryptophan; IL, interleukin; QA, quinolinic acid.

Hubbard et al., 2015). AhR signaling is considered a key component of the immune response at barrier sites and is thus crucial for intestinal homeostasis by acting on epithelial renewal, barrier integrity, and many immune cell types, such as intraepithelial lymphocytes, Th17 cells, innate lymphoid cells, macrophages, dendritic cells (DCs), and neutrophils (Lamas et al., 2018). AhR is directly activated by dietary molecules and xenobiotics. In addition, many AhR ligands are processed and inactivated by cytochrome p450 family proteins, such as Cyp1A1, which is a direct AhR transcriptional target constituting a feedback loop for AhR signaling (Schiering et al., 2017).

However, the role of microbial metabolism is preponderant in intestinal AhR activity. Indeed, the intestinal contents of germ-free or dysbiotic mice are deficient for AhR agonists (Lamas et al., 2016). Only a few commensal species able to produce AhR ligands, such as *Peptostreptococcus russellii* (Wlodarska et al., 2017) and *Lactobacillus* spp (Lamas et al., 2016; Zelante et al., 2013), have been characterized, and many likely remain to be discovered. Trp-metabolizing pathways have been

identified in some members of the human gut microbiota such as *Clostridium sporogenes*, which is able to decarboxylate Trp leading to the production of the neurotransmitter tryptamine (Williams et al., 2014). In addition, oxidative and reductive pathways have also been described in this species and lead to the production of indoleacetic acid (IAA) and indolepropionic acid (IPA), two Trp metabolites known to affect intestinal permeability and host immunity (Dodd et al., 2017; Galligan, 2018; Lamas et al., 2016) (Figure 2). Trp and indole active transporters have been identified in *E. coli*. Tryptophanase, which converts Trp into indole, is expressed in *E. coli* and lactobacilli, but the precise microbial enzymatic pathways involved in further indole processing, as well as their existence and activity in other commensal species, have yet to be described (Hubbard et al., 2015). Indole is also an interspecies signaling molecule that is able to control aspects of bacterial physiology such as antibiotic resistance, sporulation, and biofilm formation. In non-indole-producing bacteria, indole and its derivatives notably inhibit quorum sensing and modulate virulence factors (Lee et al.,



bacteria encode enzymes homologous to those of the eukaryotic KP and are thus also able to produce Kyn and downstream metabolites such as 3-hydroxyanthranilic acid (Vujkovic-Cvijin et al., 2013), which has neurotoxic effects (O'Farrell and Harkin, 2017).

### Serotonin Pathway

The neurotransmitter 5-HT is produced in the brain through the Trp hydroxylase 2 enzyme (Tph2), where it plays an important role. However, more than 90% of the body's 5-HT is produced in the gut and particularly in enterochromaffin cells (ECs), a specialized subtype of intestinal epithelial cell. This process occurs through the Trp hydroxylase 1 enzyme (Tph1), which produces 5-hydroxytryptophan (5-HTP), which is further metabolized into 5-HT. Under physiological conditions, peripheral 5-HT does not cross the blood-brain barrier.

Peripheral 5-HT triggers numerous functions in the gastrointestinal tract and is implicated in a wide range of human physiological functions by activating specific 5-HT receptors (Mawe and Hoffman, 2013). Specifically, 5-HT is an important gastrointestinal signaling molecule that conveys signals from the gut to intrinsic or extrinsic neurons and influences intestinal peristalsis and motility, secretion, vasodilatation, and the absorption of nutrients. In addition, the serotonin-selective reuptake transporter (SERT; encoded by *SLC6A4* gene), expressed in the apical and basolateral membrane of intestinal epithelial cells, acts as a sponge to remove 5-HT from the interstitial space after production by ECs. This pivotal molecule involved in the local regulation of 5-HT availability is also responsible for 5-HT reuptake in the brain.

The gut microbiota is a major actor in intestinal 5-HT production (Yano et al., 2015). Its role has been demonstrated in germ-free mice that exhibit impaired 5-HT production in the colon (but not in the small intestine) and low 5-HT concentrations in the blood. The mechanisms by which the gut microbiota modulates 5-HT production are not fully understood, but the role of SCFAs in the stimulation of Tph1 expression has been suggested (Reigstad et al., 2015). Moreover, some secondary bile acids, such as deoxycholate produced by microbial biotransformation of cholate, can also stimulate 5-HT biosynthesis (Yano et al., 2015).

### Perturbations in Tryptophan Metabolism

In the last few decades, the role of the gut microbiota has been suggested in many diseases associated with Western lifestyles, such as inflammatory bowel diseases (IBDs; regrouping Crohn disease and ulcerative colitis [UC]), irritable bowel syndrome (IBS), metabolic syndrome and associated complications (diabetes, obesity, nonalcoholic fatty liver disease [NAFLD], insulin resistance, and atherosclerosis), and neuropsychiatric traits (notably anxiety, depression, and autism). Many of these diseases are also affected by end products of Trp metabolism (Figure 3), suggesting that the effects of the microbiota are at least partially mediated by impaired Trp metabolism.

### IBD and Intestinal Immunity

The role of an altered gut microbiota is clearly demonstrated in IBD, and several seminal publications have highlighted alterations in gut Trp metabolism with potential links to intestinal microorganisms. We recently uncovered the decreased production of AhR ligands by the microbiota of IBD patients, which was

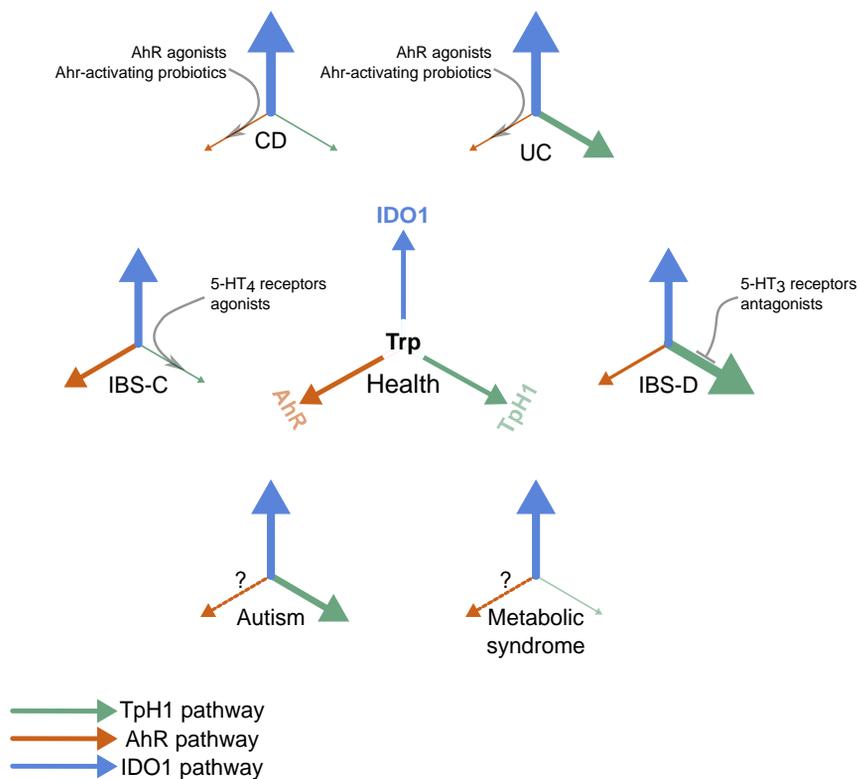
influenced by genetic factors (Lamas et al., 2016), and AhR expression in intestinal tissues was decreased compared with that in healthy subjects (Monteleone et al., 2011). Moreover, IPA is decreased in the serum of patients with UC (Alexeev et al., 2018). Overactivation of IDO1 is observed both locally in the gut (Lamas et al., 2016) and systemically, likely reflecting immune system activation. This hypothesis is supported by higher IDO1 activity in active compared with nonactive IBD patients and by the negative correlation between serum levels of Trp and C-reactive protein, a commonly used biomarker increased in response to inflammation (Nikolaus et al., 2017). The state of 5-HT pathway activation in IBD is controversial. Increased expression of the rate-limiting enzyme *Tph1* and elevated intestinal 5-HT levels have been reported in Crohn disease, while most studies have found the opposite in UC (Manocha and Khan, 2012).

These alterations in Trp metabolism have a potential role in IBD pathogenesis.

Murine studies have also shown that, in mice, AhR deficiency increases the severity of experimental colitis that is driven either by T cell transfer or chemically via administration of dextran sulfate sodium (DSS). In these models, the AhR deficiency drives colitis partially through the altered production of interleukin (IL)-22, a cytokine with a well-known effect on intestinal homeostasis (Qiu et al., 2012; Zelante et al., 2013). Our group demonstrated that the dysbiotic gut microbiota of mice deficient in caspase recruitment domain 9 (*Card9*), an IBD susceptibility gene, fails to catalyze Trp into AhR ligands, leading to reduced IL-22 release and ultimately to higher susceptibility of *Card9*<sup>-/-</sup> mice to DSS-induced colitis (Lamas et al., 2016). Some functional relevance is also seen in humans, as pharmacological activation of AhR reduces the production of the proinflammatory cytokine interferon (IFN)  $\gamma$  and increases the production of IL-22 in lamina propria mononuclear cells from IBD patients (Monteleone et al., 2011). In addition, as observed in humans with UC, IPA and indole are decreased in serum of mice with DSS-induced colitis with additional evidence indicating that oral administration of IPA has protective properties in this model system (Alexeev et al., 2018).

Alterations in the KP may also be mechanistically involved in IBD pathogenesis. *IDO1*<sup>-/-</sup> mice are more susceptible to colitis, demonstrating that IDO1 is a negative regulator of intestinal inflammation. The pathological damage associated with IDO1 deficiency is partially due to the activation of proinflammatory cytokines and a decreased number of CD4<sup>+</sup> Foxp3<sup>+</sup> regulatory T cells in the colon. However, the precise mechanisms and metabolites involved remain unknown (Takamatsu et al., 2013). Kyn is known to be an AhR agonist, but the concentration required to elicit reporter AhR activity in a hepatoma cell line casts doubt on its relevance as an AhR activator under physiological conditions (Hubbard et al., 2015). Alterations to downstream metabolic pathways leading to deficiency in anti-inflammatory metabolites, such as Kna, might be involved, but this remains to be demonstrated. In the context of IBD, abnormal signals from a dysbiotic microbiota may be drivers of a skewed KP.

The severity of chemically induced colitis is attenuated in *Tph1*<sup>-/-</sup> mice and in mice treated with the 5-HT synthesis inhibitor parachlorophenylalanine, suggesting that 5-HT



**Figure 3. Perturbations to Trp Metabolism in Diseases**

The three major pathways of Trp metabolism, differentially affected in diseases, remain tightly interconnected. Diagrams indicate repartitioning of Trp fluxes in diseases based on the available clinical data. Weights of arrows indicate strength of pathway activation. The restoration of disrupted equilibrium using molecules or probiotics represents a promising therapeutic strategy. AhR, aryl hydrocarbon receptor; IDO1, indoleamine 2,3-dioxygenase 1; TpH1, tryptophan hydroxylase 1; 5-HT, 5-hydroxytryptamine; CD, Crohn disease; UC, ulcerative colitis; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea.

worsens intestinal inflammation (Ghia et al., 2009). Moreover, deletion of SERT, leading to increased 5-HT availability, induces the exacerbation of experimental colitis (Spohn and Mawe, 2017). These proinflammatory effects might be partly driven by the activation of 5-HT<sub>7</sub> receptors on DCs. However, new clues suggest that 5-HT also exerts anti-inflammatory effects by acting on the 5-HT<sub>4</sub> receptor, with positive consequences on intestinal epithelial cell barrier functions (Spohn and Mawe, 2017).

Taken together, these data suggest that the alterations in Trp metabolism observed in IBD might have an active role in disease pathogenesis. The involvement of the microbiota is obvious in terms of the impaired ability of these microorganisms to produce AhR agonists but might also account for the exacerbated local activation of IDO and TpH1 that occurs under the direct influence of the microbiota under physiological conditions.

#### Irritable Bowel Syndrome

Although the etiology of IBS is largely unknown, the role of a dysbiotic gut microbiota in its pathogenesis is suspected, at least for some subgroups of patients (Fan et al., 2017; Hyland et al., 2014). In addition, there might be connections with impaired Trp metabolism. Kyn is increased in the serum of IBS patients (Clarke et al., 2012), and peripheral IDO1 activity is positively correlated with IBS severity (Fitzgerald et al., 2008). Alterations in gut motility, one of the key features in IBS, are linked to 5-HT dysmetabolism. TpH1 and SERT expression levels were found to be decreased in rectal biopsies from IBS patients compared with those from healthy controls (Kerckhoffs et al., 2012). In addition, 5-HT colon contents are decreased and increased in constipation- and diarrhea-predominant IBS,

respectively (Manocha and Khan, 2012). The pleiotropic effects of 5-HT are related to the diversity of its receptors that are able to trigger specific functions in specific organs. 5-HT<sub>3</sub> and 5-HT<sub>4</sub> subtypes, which are the most expressed in the gastrointestinal tract, link 5-HT to visceral nociception and motility disorders. The role of 5-HT has already been exploited as a therapeutic target with the use of 5-HT<sub>3</sub> receptor antagonists and 5-HT<sub>4</sub> receptor agonists showing some efficacy

in diarrhea- and constipation-predominant IBS, respectively (Binienda et al., 2017).

However, perturbed central serotonin action, which is also potentially modulated by gut microbiota (Figure 1), may participate in IBS pathogenesis as well (Stasi et al., 2014). The effects of the gut microbiota on 5-HT production and gut motility have been demonstrated in mice (Yano et al., 2015) and suggest that IBS pathogenesis is partly related to the dysfunctional control of 5-HT production by the microbiota.

#### Metabolic Syndrome and Obesity

In human patients with metabolic syndrome, overactivation of IDO1 has been reported with increased serum Kyn levels and a correlation between the Kyn/Trp ratio and obesity, metabolic syndrome, BMI, and blood triglycerides (Mallmann et al., 2018). Increased gene expression of *IDO1*, and of downstream enzymes of the KP such as kynureninase (KYNU), kynurenine aminotransferase (KAT), and kynurenine 3-monooxygenase (KMO) (Figure 2), has been observed in the adipose tissues of obese patients, suggesting the local activation of IDO1 (Favenec et al., 2015). However, circulating 5-HT levels are decreased in metabolic syndrome and are negatively correlated with BMI and body fat (Hodge et al., 2012). Several indole derivatives produced via Trp transformation by the microbiota may have a role in metabolic syndrome pathogenesis. Indole itself has been shown to stimulate enteroendocrine L cells to produce glucagon-like peptide-1 (GLP-1), an incretin stimulating the secretion of insulin by pancreatic  $\beta$  cells. This mechanism involves the rapid inhibition of voltage-gated K<sup>+</sup> channels stimulating GLP-1 secretion but is controlled by longer-term effects on ATP synthesis inhibition, reducing GLP-1 secretion (Chimere et al., 2014). Indole is also absorbed and metabolized to indoxyl

sulfate in the liver. During kidney failure, this metabolite accumulates, and its proinflammatory and oxidant effects are implicated in the pathogenesis of atherosclerosis, arteriosclerosis, congestive heart failure, and other cardiovascular complications that are particularly over-represented in patients with chronic kidney failure (Hung et al., 2017). The role of indoxyl sulfate in subjects with normal kidney function remains to be determined. The KP has also been implicated in atherosclerosis. In a mouse model, IDO1 deficiency reduced the development of atherosclerosis lesions through the dysregulation of IL-10 production, a phenotype reversed by the administration of Kna. In humans, high Kna levels are correlated with an unstable plaque phenotype (Metghalchi et al., 2015). The low-grade chronic inflammation that has been described in these pathologies may contribute to IDO1 activation. Overactivation of the KP is also likely to participate in the onset of insulin resistance in low-grade inflammatory situations such as obesity, depression, hepatitis C virus infection, and cardiovascular diseases. Human and experimental data suggest that xanthurenic acid and other products of the KP have deleterious effects on insulin production and release and consequences on target tissues (Oxenkrug, 2013).

Serotonin produced in the brain induces satiety, but gut-derived 5-HT produced under the direct influence of the microbiota does not cross the blood-brain barrier. However, Trp and the direct 5-HT precursor 5-HTP do cross the blood-brain barrier and thus indirectly modulate central 5-HT production and function. Moreover, peripheral 5-HT affects host metabolism independent of any central effect: gut-derived 5-HT is able to induce hypophagia and satiety (Voigt and Fink, 2015), and its level increases during fasting and stimulates lipolysis in adipose tissue and gluconeogenesis in hepatocytes, favoring impaired glycemic control (Sumara et al., 2012). Subsequently, mice with genetic or chemical ablation of Tph1 given a high-fat diet are protected against obesity, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) by a mechanism involving greater energy expenditure by thermogenic brown adipose tissue (Crane et al., 2015). However, these results might not be applicable to adult humans in whom the abundance of brown adipose tissue is low and decreases with age. In addition, obesity in humans has been associated with decreased peripheral 5-HT, suggesting a complex role in pathogenesis (Hodge et al., 2012). The role of AhR has been investigated in metabolic syndrome using mouse models, but no clear conclusion has been reached. This might be related to the multiple effects of AhR, which is expressed in various cell types (enterocytes, hepatocytes, and immune cells) involved in metabolic syndrome pathogenesis.

### Infectious Diseases

The production of Trp-derived AhR agonists, such as IAld, by the gut microbiota plays an important role in the protection against mucosal candidiasis, a fungal infection caused by *Candida* species. The underlying mechanisms involve the production of IL-22, a key cytokine in colonization resistance against fungi (Zelante et al., 2013). Similarly, the degradation of AhR ligands in response to Cyp1A1 dysregulation as well as the deletion of AhR lead to increased susceptibility to *Citrobacter rodentium* infection (Qiu et al., 2012; Schiering et al., 2017). Susceptibility to these two pathogens is reversed by the restoration of intestinal AhR activity, highlighting the importance of balanced Trp

metabolism in intestinal homeostasis and the response to infection. Independent of the production of AhR ligands, local Trp metabolism might be an adaptive component of the interactions between the host and microorganisms. Trp auxotrophy by certain pathogens, such as the intracellular bacteria *Chlamydia* and parasite *Leishmania*, is a flaw that CD4<sup>+</sup> T cells exploit to limit infection by overactivating IDO1, hijacking Trp to the KP and thus starving bacteria. Some bacteria, such as *Mycobacterium tuberculosis*, escape CD4-mediated defense by synthesizing their own Trp under stress conditions (Zhang et al., 2013). Such a mechanism has not yet been described in the gut but could be relevant for both pathogen- and microbiota-host interactions.

### Neuropsychiatric Disorders

The gut microbiota influences the brain and may be involved in neuropsychiatric disorders, partly by modulating circulating Trp availability. Although the blood-brain barrier is highly selective, Trp and Kyn cross it and have notable effects on the metabolism of neurotransmitters. Kna and QA, produced in the brain from Trp or directly from Kyn, affect brain chemistry differently by acting on glutamate receptors such as the N-methyl-D-aspartate receptor, important in memory function. Indeed, Kna and QA act respectively by decreasing or increasing extracellular levels of glutamate (Schwarcz et al., 2012), which has been shown to be involved in anxiety and stress-related disorders. In this context, IDO1 activation in the periphery, notably in the case of inflammation, may remotely affect these cerebral processes, although the mechanisms involved remain unclear (Schwarcz et al., 2012). Recently, the pathogenesis of autism spectrum disorder (ASD), one of the most serious neurodevelopmental conditions worldwide, has been suggested to involve an altered gut microbiota. Moreover, patients with ASD display altered Trp metabolism characterized by reduced plasma and urine levels of Trp (Kałużna-Czaplińska et al., 2017), high IDO1 activity (assessed by Kyn/Trp ratio) (Lim et al., 2016), and high 5-HT blood levels (Muller et al., 2016).

An experimental survey in mice mimicking autism syndrome reported a 50% reduction in small and large intestine mucosal 5-HT with some correlations among 5-HT levels, intestinal transit time, and the abundance of certain bacterial taxa such as *Blautia* (Golubeva et al., 2017). These results are in line with the frequent constipation observed in patients with ASD. Thus, if low intestinal production of 5-HT is confirmed in humans with ASD, the origin of the increased 5-HT blood levels observed in these patients must be identified outside of the gut. In addition to 5-HT, other Trp metabolism pathways might participate. Total neuroprotective metabolites such as picolinic acid (PA), a metabolite of the KP, are decreased in ASD patients despite an excess of QA production (Lim et al., 2016). Moreover, AhR signaling might also be involved, as polymorphisms in the gene encoding *AhR nuclear translocator (ARNT)*, which binds and facilitates AhR functions, are associated with ASD severity (Fujisawa et al., 2016).

Emerging evidence implicates the microbiome-gut-brain axis in depression as well. Decreased availability of 5-HT in the brain is a key feature in the pathogenesis of depression. In the case of IDO1 pathway overactivation, such as that occurring in chronic inflammatory diseases or in patients treated with interferon for hepatitis C, Trp is massively diverted to the production of Kyn,

causing a deficiency in brain Trp and in 5-HT production, subsequently leading to depression. A similar hypothesis exists regarding depression in obesity, a condition also characterized by chronic inflammation and IDO1 activation (Chaves Filho et al., 2018). A comparable effect is observed in the case of Trp deficiency due to low dietary intake, supporting the role of Trp pool depletion in depression.

### Tryptophan Metabolism: From a Disrupted Equilibrium to Clinical Opportunities

Since Trp metabolism is affected in pathological situations, the use of Trp and its metabolites as biomarkers to support diagnosis and prognosis and to orientate therapeutic choices is attractive. For example, plasma levels of Trp and Kna predict adverse cardiovascular outcomes in patients hospitalized for acute myocardial infarction (Metghalchi et al., 2015). This approach can likely be expanded and applied to the other diseases described above.

In addition to being biomarkers, the biological effects of Trp metabolites and their alterations in disease suggest that they may be therapeutic targets. This is directly achieved by the use of Trp metabolites, or by targeting their receptors, or by indirectly manipulating the gut microbiota. For example, the administration of *Lactobacillus*, which naturally produces AhR agonists, improves colitis severity in mice with genetically induced dysbiosis, suggesting potential therapeutic applications in IBD (Lamas et al., 2016). Similarly, *Lactobacillus reuteri*, through the production of the AhR agonist indole-3-lactic acid, is able to reprogram intraepithelial CD4<sup>+</sup> T cells into immunoregulatory CD4<sup>+</sup>CD8 $\alpha\alpha$ <sup>+</sup> T cells (Cervantes-Barragan et al., 2017).

In situations where IDO is over-activated, decreased gastrointestinal Trp availability may contribute to lower production of AhR agonists by the gut microbiota. This is the case in settings of intestinal inflammation, such as DSS-induced colitis in mice in which dietary Trp supplementation alleviates colitis severity through restoration of AhR ligand production by the gut microbiota (Islam et al., 2017). Similar protective effects have been reported in a porcine colitis model (Kim et al., 2010).

This type of approach can be expanded to other inflammatory conditions such as multiple sclerosis, in which decreased levels of circulating AhR agonists have been observed. In an encephalomyelitis mouse model, supplementation with Trp or Trp-derived AhR agonists has been shown to potentiate IFN suppressive effects in an AhR-dependent manner and to limit CNS inflammation (Rothhammer et al., 2016). The pathological role of indoxyl sulfate in kidney diseases might also be a feasible target, as suggested by a recent study showing that manipulation of the gut microbiota with diet and genetically modified bacteria controls indoxyl sulfate levels (Devlin et al., 2016). Manipulation of the IDO1 and 5-HT pathways in the gut using microbiota-based approaches is also attractive, but better knowledge of the microorganisms and mechanisms involved is needed for further development.

To date, a large part of Trp-metabolizing microorganisms, as well as the associated biochemical pathways, remains to be characterized. Factors complicating this task include the diversity of Trp-derived bioactive molecules, the variety of microorganisms involved in their transformation, and the partial overlap of microbial and host pathways. These impediments

can be addressed with high-sensitivity and/or high-throughput methods, which are constantly under development. Combining different methods, particularly metabolomics, with metagenomics and/or metatranscriptomics seems a particularly promising strategy to identify microbes and microbial genes involved in the modulation of Trp metabolism. Once identified, one can imagine using natural microbes; genetically engineered bacteria; or, more directly, microbial products to modulate Trp metabolism in a therapeutic context. This type of intervention might be used either alone or more likely in combination to target several pathways and with concomitant modulation of Trp intake.

### Concluding Remarks

Trp metabolism has a central role in physiology and physiopathology. The major pathways described above, specifically the 5-HT, Kyn, and AhR pathways, are differentially affected in diseases but remain tightly interconnected. Moreover, intestinal Trp metabolism in these pathways is directly or indirectly controlled by the microbiota (Figure 3). Trp metabolism in the gut is therefore an actionable actor from a therapeutic perspective, using either molecules targeting a specific pathway or exploiting microorganisms manipulating Trp metabolism as probiotics. However, the complexity of microbiota-host interactions and the sophistication of the diseases and models studied demand further investigation to refine targets and therapeutic interventions.

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### DECLARATION OF INTERESTS

Patents related to this work (H.S.): EP 15306303.7; EP16306300.1. H.S. is a co-founder of Nextbiotix.

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