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REVIEW

Tryptophan-derived serotonin-kynurenine balance in immune activation and intestinal inflammation

Sabah Haq[1,2] | **Jensine A. Grondin**[1,2] | **Waliul I. Khan**[1,2,3]

[1]Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada

[2]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

[3]Laboratory Medicine, Hamilton Health Sciences, Hamilton, Ontario, Canada

Correspondence
Waliul I. Khan, Farncombe Family Digestive Health Research Institute, McMaster University Health Sciences Centre, Room 3N7, 1280 Main Street West, Hamilton, ON, Canada.
Email: khanwal@mcmaster.ca

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Abstract

Endogenous tryptophan metabolism pathways lead to the production of serotonin (5-hydroxytryptamine; 5-HT), kynurenine, and several downstream metabolites which are involved in a multitude of immunological functions in both health and disease states. Ingested tryptophan is largely shunted to the kynurenine pathway (95%) while only minor portions (1%–2%) are sequestered for 5-HT production. Though often associated with the functioning of the central nervous system, significant production of 5-HT, kynurenine and their downstream metabolites takes place within the gut. Accumulating evidence suggests that these metabolites have essential roles in regulating immune cell function, intestinal inflammation, as well as in altering the production and suppression of inflammatory cytokines. In addition, both 5-HT and kynurenine have a considerable influence on gut microbiota suggesting that these metabolites impact host physiology both directly and indirectly via compositional changes. It is also now evident that complex interactions exist between the two pathways to maintain gut homeostasis. Alterations in 5-HT and kynurenine are implicated in the pathogenesis of many gastrointestinal dysfunctions, including inflammatory bowel disease. Thus, these pathways present numerous potential therapeutic targets, manipulation of which may aid those suffering from gastrointestinal disorders. This review aims to update both

Abbreviations: 3-HAA, 3-hydroxyanthranilic; 3-HK, 3-hydroxykyneurinine; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; 5-HTR, 5-HT receptor; AADC, aromatic L-alpha amino acid decarboxylase; AANAT, aryl alkylamine N-acetyltransferase; AhR, aryl hydrocarbon receptor; ALDH, aldehyde dehydrogenase; AMP, antimicrobial peptide; APC, antigen-presenting cell; cAMP, cyclic adenosine mono-phosphate; CARD9, Caspase recruitment domain-containing protein 9; CD, Crohn's disease; CNS, central nervous system; DC, dendritic cell; DNBS, dinitrobenzenesulfonic acid; DSS, dextran sulphate sodium; EC, enterochromaffin; Foxp3, forkhead box P3; GF, germ-free; GI, gastrointestinal; GPCR, G-protein-coupled receptor; HIOMT, hydroxyindole-O-methyltransferase; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IDO, indoleamine 2,3 dioxygenase; IFN, interferon; IL, interleukin; ISS-ODN, oligodeoxynucleotides containing immunostimulatory sequences; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; KYNA, kynurenic acid; MAO-A, monoamine oxidase A; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; NK, natural killer; NOD1, nucleotide-binding oligomerization domain-containing protein 1; OLFR, olfactory receptor; PI3K, phosphatidylinositol-3-kinase; SERT, serotonin reuptake transporter; SSRI, selective serotonin reuptake inhibitor; TDO, tryptophan 2,3 dioxygenase; TGF-β, transforming growth factor-β; TLR, toll-like receptor; TNBS, trinitrobenzene sulphonic acid; TNF-α, tumor necrosis factor α; Tph, tryptophan hydroxylase; T_{reg}, regulatory T cells; UC, ulcerative colitis; WT, wild-type.

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the role of 5-HT and kynurenine in immune regulation and intestinal inflammation, and analyze the current knowledge of the relationship and interactions between 5-HT and kynurenine pathways.

KEYWORDS
immune cells, intestinal inflammation, kynurenine, microbiota, serotonin

1 | INTRODUCTION

Metabolism of the essential aromatic amino acid, tryptophan, plays an important role in intestinal mucosal homeostasis and in the pathophysiology of inflammation. The endogenous metabolism of tryptophan mainly occurs along the kynurenine and serotonin (5-hydroxytryptamine or 5-HT) pathways.[1] The vast majority (95%) of this dietary tryptophan is shunted to the kynurenine pathway. Only a minor portion (1%–2%) of the dietary tryptophan enters the 5-HT pathway and, ultimately, contributes to 5-HT production. In addition, a small portion (4%–6%) of ingested tryptophan is subjected to bacterial degradation in the gut lumen, primarily generating indole and other related compounds.[2] The downstream agents of the kynurenine pathway play a number of physiological roles ranging from fortifying immunological defenses and regulating gut motility to maintaining aspects of central nervous system (CNS) functioning.[2] Though often thought of as a distinct neurotransmitter of the CNS, regulating mood, anxiety and behaviour,[3,4] 5-HT, a bioamine, is synthesized in both CNS and peripheral tissues, particularly within the gastrointestinal (GI) tract. These gut-derived secretions account for approximately 95% of the body's total 5-HT.[5,6] 5-HT has diverse effects within the body including roles as a hormone and neurotransmitter, as an autocrine and paracrine factor when acting upon its array of receptors, and as an element of intracellular signaling. Additionally, over the last 20 years, 5-HT has been implicated in GI function, particularly with regard to motility, peristaltic activity, and intestinal secretions.[7–9] 5-HT also has substantial influence on inflammatory pathways and has significant influence in the pathogenesis of a number of GI disorders including inflammatory bowel disease (IBD).[5,10] In recent years, the involvement of the other tryptophan metabolites, particularly those generated in the kynurenine pathway, in intestinal immune homeostasis, inflammation and GI disorders such as IBD and colorectal cancer has been an area of considerable investigation.[2,11]

The gut microbiota is another critical component in the regulation of intestinal immune response and inflammation. The aforementioned endogenous tryptophan metabolites have a significant impact on gut microbial composition, on the host-microbial interface and on immune-microbe interactions. Reciprocally, the gut microbiota also influences the endogenous metabolism of tryptophan via 5-HT and kynurenine pathways.[12] Both pathways have been studied extensively with regard to the pathophysiology of depression; however, their roles in intestinal immune regulation and inflammation have yet to be fully elucidated.[1] There is some evidence suggesting that 5-HT and kynurenine pathway metabolites interact with each other by influencing rate-limiting enzymes, receptors, or simply the availability of tryptophan.[1,2,13] Though the roles that 5-HT-kynurenine interactions play in the pathogenesis of gut inflammatory disorders are not yet clear, the clinical implications of these relationships cannot be ignored. Therefore, this review will explore the recent developments of effects and interactions of the 5-HT and kynurenine pathways in the modulation of immune cells, gut microbiota, and intestinal inflammation.

2 | SYNTHESIS AND METABOLISM OF 5-HT AND KYNURENINE IN THE GUT

The generation of 5-HT is concentrated in the nervous system including the brain and enteric neurons, and the GI tract. Within the GI tract, the enterochromaffin (EC) cells of the epithelial layer produce the vast majority of the body's 5-HT.[5,6] Neuron- and EC cell-derived 5-HT represent not only locationally different sources of 5-HT but also exhibit biochemical differences in production as well.[5,6,14] Of the two enzymatic isoforms, tryptophan hydroxylase (Tph) 2 acts within both central and enteric neurons and controls the production of 5-HT particularly within the raphe nuclei of the hindbrain.[15–17] Tph1, in contrast, is localized largely to EC cells within the gut.[15,18] Though both enteric neurons and EC cells contribute to "gut-derived" 5-HT synthesis and secretion, 90% of this organ's 5-HT production is accounted for by the EC cells of the epithelial layer and only a minor contribution, of approximately 10% is derived from underlying enteric neurons.[18–20] Tph1 has also been found in fibroblasts, pancreatic and respiratory tissue, as well as the pineal gland, where it plays an upstream role in the production of melatonin.[14,15,21]

The process of 5-HT biosynthesis begins with the ingestion of tryptophan-containing substances. Within the gut, dietary tryptophan is taken up by enterocytes, both apically via the B^0AT1 (Solute Carrier 6A19, SLC6A19) epithelial amino acid transport system and basolaterally via the aromatic amino acid transporter TAT1 (Slc16a10) protein.[2] Within the EC cells of the gut, Tph1 catalyzes the 5-hydroxylation of this dietary-derived tryptophan to 5-hydroxytryptophan (5-HTP).[22] In EC cells, aromatic L-alpha amino acid decarboxylase (AADC), along with the cofactor, pyridoxal-5′-phosphate (the active form of vitamin B6), rapidly converts 5-HTP to 5-HT via decarboxylation (Figure 1).[2,22]

Once synthesized, 5-HT is sequestered via vesicular monoamine transporter-1 and -2 into large dense core storage vesicles and synaptic-like microvesicles located at the base of the cell.[2,14] Here, it is held until it is released into extracellular space, either apically to the gut lumen or basolaterally to the lamina propria, where it largely exerts paracrine effects on enteric neurons and can enter the bloodstream.[15,23,24] Enteroendocrine cells, of which EC cells are a subtype, act as physical and chemical sensory transducers within the gut via their apical microvilli, which extend into the lumen.[25,26] Any excess 5-HT in the EC cell not stored in the vesicles is subjected to oxidative deamination by monoamine oxidase A (MAO-A), resulting in 5-hydroxyindoleacetaldehyde and further metabolized by aldehyde dehydrogenase (ALDH) producing 5-hydroxyindole acetic acid (5-HIAA), which is mainly excreted in the urine (Figure 1).[22,27] Upon its release from the grips of EC cells, the termination of 5-HT activity can occur in a number of ways including exerting influence on intracellular signaling by impacting one of several 5-HT receptors (5-HTRs),[3,5] having a direct impact on luminal bacteria,[28] or being subjected to reuptake by platelets, neighboring epithelial or immune cells via serotonin reuptake transporter (SERT).[5,29,30]

Previously thought to be restricted to the pineal gland of the brain, 5-HT in the gut can also be shunted into the melatonin pathway.[31] Melatonin is known mainly as a key photoregulated hormone involved in controlling the sleep-wake cycle/circadian rhythm and is increasingly being investigated within the context of the gut as well as for its influence on immunological processes.[31,32] Extra-pineal melatonin synthesis can occur in the EC cells of the gut where 5-HT can be directly metabolized to N-acetyl-5-hydroxytryptamine by local aryl alkylamine N-acetyltransferase (AANAT) and eventually to melatonin or N-acetyl-5-methoxytryptamine by hydroxyindole-O-methyltransferase (HIOMT) (Figure 1).[2,14,31] This extra-pineal melatonin is considered

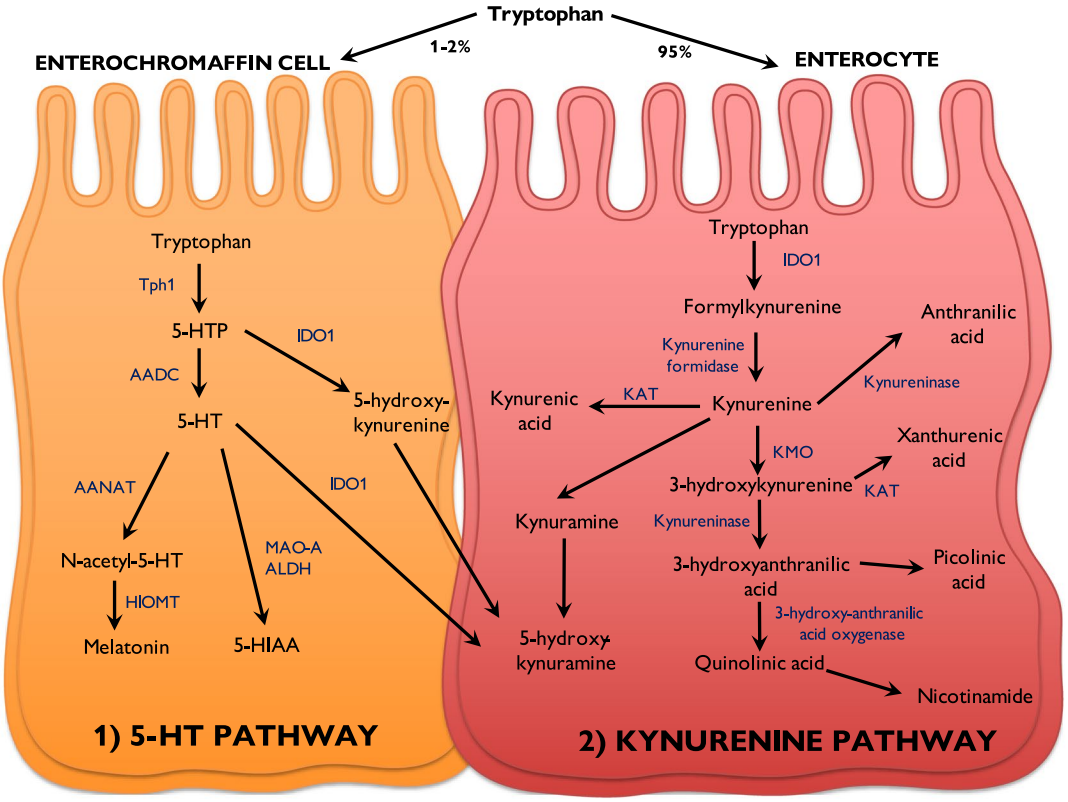

FIGURE 1 Endogenous tryptophan metabolic pathways. In the gut, about 1%–2% of dietary tryptophan is metabolized through Tph1 mediated 5-HT pathway. About 95% of dietary tryptophan is metabolized through IDO1 mediated kynurenine pathway. (1) 5-HT pathway of tryptophan metabolism occurs mainly in the enterochromaffin cells. (2) Kynurenine pathway of tryptophan metabolism occurs mainly in the intestinal epithelial cells (shown) and antigen-presenting cells (not shown). 5-HT can enter portions of the kynurenine pathway via conversion to 5-hydroxykynuramine by IDO1. 5-HTP can also be converted to 5-hydroxykynurenine by IDO1. Key enzymes are represented in blue

an independent reservoir from the melatonin produced in the pineal gland.[33]

Though 5-HT is ultimately derived from ingested tryptophan, the vast majority (95%) of this dietary tryptophan is shunted to the kynurenine pathway.[2] Within this pathway, peripheral breakdown of tryptophan begins with either of the rate-limiting enzymes tryptophan 2,3 dioxygenase (TDO), which is largely present in hepatocytes, or indoleamine 2,3 dioxygenase (IDO). IDO, a monomeric reductase, is extensively distributed throughout a number of peripheral tissues including the gut.[34] Two isoforms of this enzyme exist, IDO1 and IDO2, with IDO1 being the most extensively studied and found in intestinal epithelial cells and immune cells such as macrophages, dendritic cells (DCs), and B cells.[2] TDO and IDO2 are not expressed in the gut.[35] Catalyzing the initial step of the kynurenine pathway, IDO1 converts L-tryptophan to N-formylkynurenine. N-formylkynurenine is then converted to kynurenine by kynurenine formidase. From here, kynurenine can be metabolized to kynuramines, to kynurenic acid (KYNA) via kynurenine aminotransferase (KAT), to 3-hydroxykyneurinine (3-HK) via kynurenine 3-monooxygenase (KMO), or to anthranilic acid via kynureninase.[2] 3-HK is further converted into xanthurenic acid, 3-hydroxyanthranilic (3-HAA), quinolinic acid, and, ultimately, nicotinamide adenine dinucleotide (NAD+) (Figure 1).[2,35]

Unlike 5-HT, kynurenine and its downstream metabolites do not have unique kynurenine receptors but share common receptors with other neurotransmitters and endogenous ligands. Kynurenine is an endogenous ligand of the aryl hydrocarbon receptor (AhR) which affects the metabolism of xenobiotics and the pathogenesis of cancer. However, kynurenine does not activate AhR under normal conditions due to very low in vivo concentrations.[36] Quinolinic acid is an excitotoxic *N*-methyl-D-aspartate receptor agonist but is also pro-oxidant with immunomodulatory actions. KYNA, on the other hand, acts as a neuroprotective *N*-methyl-D-aspartate receptor antagonist and α7-nicotinic cholinergic agonist. KYNA is also an agonist of the orphan G-protein-coupled receptor 35 (GPCR35).[37] Alterations in the kynurenine pathway affecting substrate availability and several downstream metabolites have been implicated in a number of conditions, including depression and other CNS conditions, HIV, and gut disorders including irritable bowel syndrome (IBS) and IBD.[1,2,38–41]

3 | 5-HT AND THE KYNURENINE PATHWAY IN IMMUNE REGULATION

3.1 | 5-HT and immune cells

Several investigations over the last 20 years have revealed that 5-HT has both direct and indirect effects on immune cells. Peripheral 5-HT, produced from EC cells, is taken up by SERT and stored in the dense granules of platelets which provides the largest source of 5-HT for immune cells.[24,42] Apart from this, significantly smaller sources of 5-HT are synthesized by immune cells themselves, including mast cells, monocytes, and T cells.[43] The presence of several types of 5-HTRs and SERT on cells of innate and adaptive immunity supports the enormous influence 5-HT signaling has on the immune system. Mammalian immune cells express 7 families with 15 different subtypes of 5-HTRs (5-HTR1-7) on their plasma membrane.[25,44] All 5-HTRs belong to the GPCR superfamily except 5-HTR3 which is a Cys-loop ligand-gated ion channel.[25,44] GPCRs 5-HTR1 and 5-HTR5 couple negatively to adenylyl cyclase and downregulate cyclic adenosine mono-phosphate (cAMP) production upon activation. In contrast, stimulation of 5-HTRs 4, 6 and 7 promote cAMP activity by activation of adenylyl cyclase.[25,45] Activation of 5-HTR2 increases cytosolic Ca^{2+} by the upregulation of inositol triphosphate and diacylglycerol pathways.[25,45] Activation of 5-HTR3, which is a non-selective cation channel triggers rapid depolarization of the plasma membrane due to Ca^{2+} and Na^{+} influx and K^{+} efflux.[25,45] Furthermore, stimulation of these 5-HTRs ultimately regulate downstream signaling cascades via phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) and mitogen associated protein kinase dependent pathways.[18] The close proximity of EC cells to $CD3^{+}$ and $CD20^{+}$ lymphocytes in the lamina propria provides further evidence that 5-HT synthesized from EC cells may directly influence immune cells.[46] Table 1 summarizes the expression of 5-HTRs on the different immune cells of the body.

5-HT has both inhibitory and stimulatory effects on monocytes and macrophages depending on the dose and receptors present. Monocytes express 5-HTRs, SERT, as well as Tph1 and MAO, highlighting the influence 5-HT can have on these cells.[47] 5-HT also regulates interferon-γ (IFN-γ)-induced phagocytosis in bone marrow-derived macrophages. Notably, this effect depends on the concentration of IFN-γ. At high IFN-γ doses, 5-HT inhibits macrophage phagocytosis, whereas, at a low physiological concentration of IFN-γ, 5-HT increases this process.[48] When treated with 5-HT, mouse peritoneal macrophages exhibit increased production of pro-inflammatory cytokines in a nuclear factor kappa-light-chain enhancer of activated B-cells (NF-κB)-dependent manner along with an upregulation of phagocytosis via 5-HTR1A in a dose-dependent manner.[49–51] 5-HT has also been shown to increase the release of superoxides from macrophages and modulate the process of macrophage polarization.[52] Interestingly, in contrast to the previously mentioned findings suggesting that 5-HT increases the production of pro-inflammatory cytokines, Casas-Engel et al. reported opposing results; in these studies, 5-HT was

TABLE 1 Family, subtype and expression of 5-HT receptors on immune cells

5-HTR family and subtypes	Immune cell type
5-HTR1A	Mast cells, eosinophils, monocytes, macrophages, NK cells, T cells, B cells
5-HTR1B	Eosinophils, macrophages, DCs, T cells
5-HTR1D	Unknown
5-HTR1E	Eosinophils, monocytes, macrophages, DCs
5-HTR1F	Unknown
5-HTR2A	Monocytes, macrophages, DCs, eosinophils, NK cells, T cells, B cells
5-HTR2B	Monocytes, macrophages, DCs, eosinophils, NK cells
5-HTR2C	Macrophages, NK cells, T cells
5-HTR3	Monocytes, macrophages, DCs, T cells, B cells
5-HTR4	Monocytes, macrophages, DCs
5-HTR5	Unknown
5-HTR6	Eosinophils
5-HTR7	Neutrophils, monocytes, macrophages, DCs, T cell, B cells

Source: The table has been adopted from Shajib et al.,[25] Wan et al.[44] and Herr et al.[47]

shown to inhibit the lipopolysaccharide-induced release of pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin (IL) 12p40. Moreover, the authors also found that 5-HT upregulates the expression of M2 polarization–associated genes through activating 5-HTR2B and 5-HTR7, and reduces the expression of M1-associated genes.[53] These contradictory findings highlight the importance of receptor subtypes in the modulation of macrophage function by 5-HT.

Natural killer (NK) cells respond to the effects of 5-HT by their surface expression of 5-HTR1A and 5-HTR2A-C.[54] In the early 1990s, indirect effects of 5-HT on NK cells were identified through 5-HT mediated regulation of monocytes. 5-HT modulates the interaction between monocytes and NK cells resulting in an increase of NK cell functions including enhanced IFN-γ production and cytotoxicity.[42,55,56] In recent years, further analysis of the mechanisms of monocyte-NK cell interactions revealed that 5-HT protects NK cells from monocyte mediated apoptosis, oxidative damage and, thus, helps in maintaining the functions of NK cells despite the presence of inhibitory monocytes.[57] Increased concentration of serum 5-HT was linked with enhanced NK cell cytotoxicity.[54] Furthermore, major depressive disorder patients treated with selective serotonin reuptake inhibitors (SSRIs) for short term had increased NK cell cytotoxicity where as long term treatment resulted in increased NK cell proliferation.[58] 5-HT treatment of NK cell line, KHYG-1, at physiological concentrations enhanced their migratory behavior without affecting cytotoxicity.[54] These findings suggest that the effect of 5-HT on NK cell function and behavior is highly dependent on the concentration of 5-HT.

The differentiation of monocytes to DCs is also modulated by 5-HT.[25] Following this differentiation, 5-HT plays important roles in the functional capacity of DCs as supported by the presence of 5-HTRs in mature and immature DCs.[59] In mature DCs, activation of 5-HTR3, 5-HTR4, and 5-HTR7 enhances the release of the cytokines IL-1β, IL-6, and IL-8, and reduces the secretion of IL-12 and TNF-α.[59,60] 5-HT has also been shown to induce the production of chemokines by DCs. Further, 5-HT treated DCs resulted in a preference for the Th2 polarization of naïve $CD4^+$ T cells.[60] Our laboratory has established a critical role of 5-HT in increasing the production of pro-inflammatory cytokine IL-12 from DCs via activation of the NF-κB pathway and sequential $CD4^+$ T cell activation in relation to generation of gut inflammation.[61]

Using a *Tph1*$^{-/-}$ mouse model which has a marked reduction in gut 5-HT, Duerschmied and colleagues demonstrated that gut-derived 5-HT promotes rolling and adhesion and, thus, impacts the recruitment of neutrophils to sites of acute inflammation.[47,62] 5-HT also plays a critical role in the migration and recruitment of other innate immune cells such as eosinophils via 5-HTR2A and mast cells via 5-HT1A to sites of acute inflammation.[63,64] Further, 5-HT promotes angiogenesis at the site of inflammation by stimulating the production of vascular endothelial growth factor in a PI3K/Akt/mTOR-dependent pathway in endothelial cells (Figure 2).[65]

In addition to its wide array of effects on innate immune cells, 5-HT also regulates the function of adaptive immune cells directly through 5-HTRs expressed on T and B lymphocytes, and indirectly by influencing the actions of antigen-presenting cells (APCs) (Figure 2). B cells

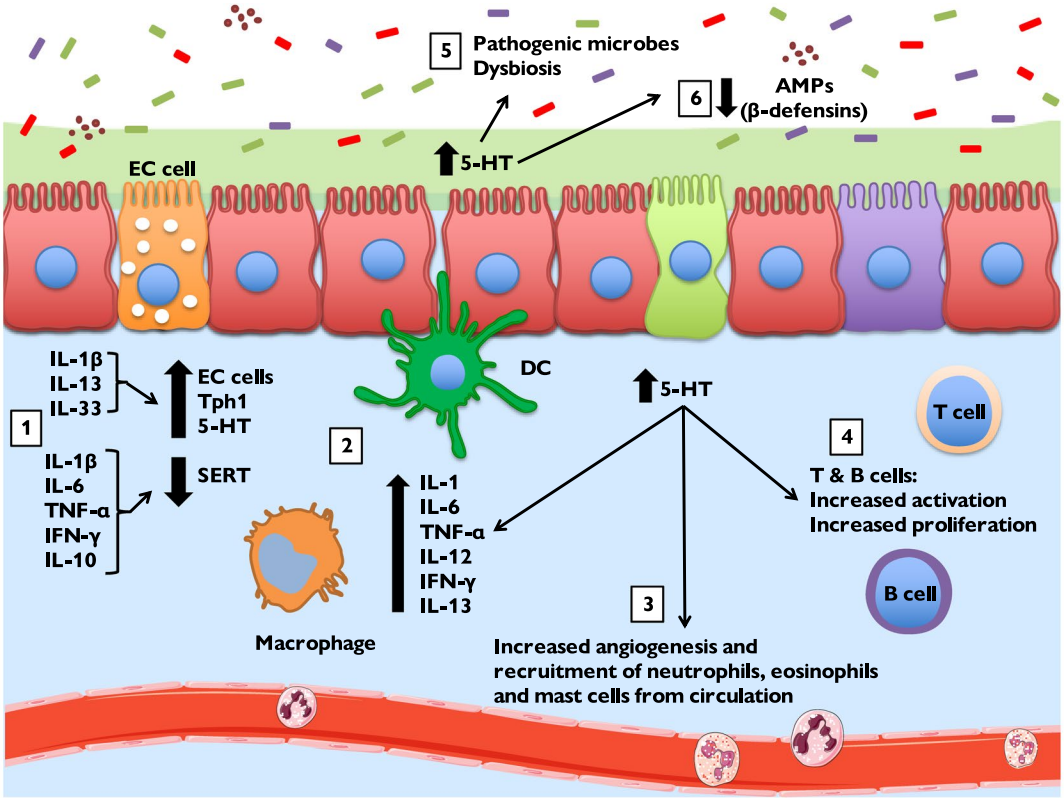

FIGURE 2 Schematic representation of the effect of mucosal 5-HT in the immune response and intestinal inflammation. This diagram shows the state of the mucosal immune system and microbiota in the context of increased mucosal 5-HT signaling. (1) Pro-inflammatory cytokines IL-1β, TNF-α, IL-6, IFN-γ, and anti-inflammatory cytokine IL-10, at low levels, reduce the expression of SERT. Cytokines IL-1β, IL-33, and IL-13 enhance the secretion of 5-HT from EC cells. IL-13 increases EC cell number and Tph1 mRNA. Elevated mucosal 5-HT during intestinal inflammation leads to: (2) Increased expression of cytokines IL-1, IL-6, TNF-α, IL-12, IFN-γ, and IL-13 from activated macrophages and dendritic cells (DCs). (3) Increased angiogenesis and recruitment of neutrophils, eosinophils, and mast cells from the circulation to sites of inflammation. (4) T and B cell activation and proliferation. (5) Development of a colitogenic microbiota. (6) Decreased production of anti-microbial peptides (AMPs) such as β-defensins

express several 5-HTRs as well as SERT.[25,47] 5-HT increases mitogen-stimulated B cell proliferation via the 5-HTR1A.[66] In this vein, long term use of SSRI in patients with major depressive disorders has been reported to increase the number of circulating B lymphocytes by ~30%.[67] Serafeim et al. demonstrated that active uptake of 5-HT via SERT drives apoptosis of Burkitt lymphoma cells, whereas the SSRIs fluoxetine, paroxetine, and citalopram blocked this action.[68] In a follow-up study, it was seen that higher doses of SSRIs trigger rapid and extensive apoptosis by inhibiting DNA synthesis. Interestingly, healthy peripheral blood mononuclear cells and tonsilar B cells are resistant to SSRI-triggered apoptosis.[69] These findings open the discussion of whether the 5-HT-altering SSRI antidepressants can be used for the targeted therapy of Burkitt lymphoma cells without affecting the function of normal B cells.

5-HT also acts as an endogenous hormone which stimulates T cell proliferation and activation via autocrine and paracrine signaling.[25] T lymphocytes express 5-HTRs along with Tph1, SERT and MAO.[47] Exogenous 5-HT induces phosphorylation of extracellular signal-regulated kinase-1 and -2 and IκBα in naive T cells, which is blocked by the 5-HTR7 antagonist SB269970 resulting in early T cell activation.[70] Additional studies in both human and mouse T cells provide further evidence that stimulation of 5-HTR1A, 5-HTR1B, 5-HTR2A, and 5-HTR3 promote T cell proliferation, differentiation, and function, demonstrating the immunostimulatory role of 5-HT.[71–74] It should be noted, however, that despite these findings, some researchers have recorded immunosuppressive roles of 5-HT in the context of T cell biology.[75,76] It is important to acknowledge that most of the studies on T cells and 5-HT do not differentiate between the different subtypes of T cells. Thus, it can be reasoned that the contradictory findings of 5-HT-induced immune activation and suppression may be due to the differences in 5-HTRs expression by T cell subtypes.[77] This hypothesis is further supported by the fact that $CD8^+$ T cells have significantly greater expression of Tph1 and MAO-A mRNA as well as higher production of 5-HT compared to

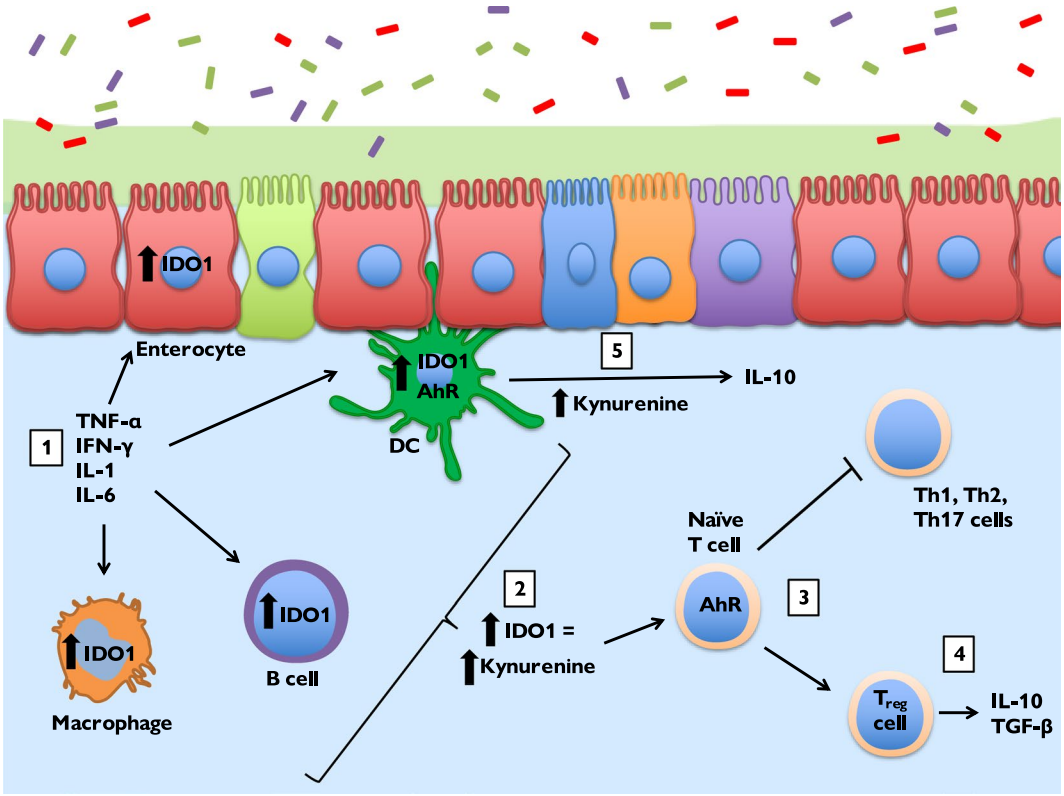

FIGURE 3 Schematic representation of the effect of kynurenine in the immune response and intestinal inflammation. IDO1 and kynurenine represent a local compensatory mechanism in inflammation in order to suppress overactive and damaging T cell activity and boost the anti-inflammatory T_{reg} cells in the colon. (1) Pro-inflammatory cytokines TNF-α, IFN-γ, IL-1 and IL-6 upregulates expression of IDO1 in intestinal epithelial cells, antigen-presenting cells like macrophages, dendritic cells (DCs) and B cells. (2) Upregulated and activated IDO1 increases the synthesis of kynurenine from tryptophan. (3) Kynurenine activates AhR in the naïve T cells that cause the differentiation toward T_{reg} cells and inhibits the production of Th1, Th2 and Th17 cells. (4) Activated T_{reg} cells secrete anti-inflammatory cytokines IL-10 and TGF-β. (5) Activation of AhR in the DCs by kynurenine increases the secretion of IL-10

$CD4^+$ T cells isolated from the spleens of male C57BL/6 mice.[78]

Melatonin, a downstream metabolite of the 5-HT pathway, is an immune modulator with both pro- and anti-inflammatory roles.[79] The role of melatonin in the regulation of the immune system and in inflammation is beyond the scope of this review and is elaborately described by Hardeland et al.[79]

3.2 | The kynurenine pathway and immune cells

The kynurenine pathway and its various metabolites have a complex relationship with the immune system.[80] Under intense immune activation or inflammatory insult, local IDO upregulation occurs. This upregulation has been shown to be grossly intertwined with immune regulation, antimicrobial activities, tumor surveillance, and both innate and adaptive defense.[1,80,81] Though other pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1, have been shown to play a role, IDO upregulation is principally induced by IFN-γ (Figure 3).[40,81–83] IDO's impact on immune function is largely predicated on two distinct theories; the local extracellular degradation and subsequent depletion of tryptophan, and the balance of downstream pro- and anti-inflammatory kynurenines.[80,84,85] Depletion of local tryptophan not only limits substrate availability for other pathways including 5-HT production[40,82,86] but also provides less of this amino acid for usage by luminal bacteria, viruses, and parasites and, thus, alters innate host defense.[87,88] In addition, the diminishment of this substrate also affects the growth, proliferation, and survival of host cells, including immune T lymphocytes and NK cells.[81]

IDO is present in cells of the immune system, largely APCs, including DCs, B cells, and macrophages, as well as epithelial cells and tumor cells.[80,81] Though IDO is confined to the intracellular microenvironment, secreted kynurenines and the aforementioned local depletion of tryptophan can greatly affect neighboring cells.[89] IFN-γ released from stimulated DCs can act in an autocrine or paracrine manner to initiate IDO expression.[81] The subsequent breakdown of tryptophan within the DC and

the local nutrient deprivation this imposes can also affect T cell proliferation, initiate the highly conserved "integrated stress response" program, and sensitize these cells to apoptotic pathways.[2,89–91] These effects support peripheral tolerance,[81] help fight local damage, and provide immunosuppression in cancerous tissue.[2,80,92] Activation of the AhR in the DCs by kynurenine increases the secretion of IL-10 (Figure 3).[93] Local conditions created by IDO via DCs also have been shown to activate regulatory T cells (T_{reg})[94] and impact the mTOR pathway, a critical pathway in immune regulation and widely thought of as a connector between metabolic processes and the functions of the immune system.[80,93] The mTOR signaling pathway which also regulates cell growth and autophagy can be inhibited by IDO-mediated depletion of tryptophan.[95] mTOR regulates the expression of the transcription factor, forkhead box P3 (Foxp3) in T_{reg} cells and mTOR inhibitors, which promote autophagy, also promote the suppressive function of T_{reg} cells and antagonize the $T_{effector}$ cell-like phenotype.[96] Further studies on the impact of 5-HT, kynurenine and their derivatives on the balance of immune cell differentiation and autophagy will help elucidate how endogenous tryptophan catabolism regulates basic cellular functions and contributes to the maintenance of cell homeostasis.

IDO itself and the downstream metabolites of the kynurenine pathway may also directly impact immune cells. For instance, kynurenine promotes T_{reg} differentiation directly by acting as a ligand for the xenobiotic-sensing transcription factor, AhR.[97] Along with neurotoxic properties, the downstream metabolites quinolinic acid, 3-HAA, 3-HK, and NAD have been shown to play a particular role in immunomodulation and in influencing apoptotic pathways.[41,98] IDO, kynurenine and its downstream metabolites suppress DC, monocytes, macrophages, and NK cell activity, and promote apoptosis in T cells.[41,99,100] 3-HAA inhibits PI3K/Akt/mTOR and NF-κB signaling pathways and decreases the production of pro-inflammatory cytokines, IL-6 and TNF-α from macrophages.[101] The immunosuppressive effect of 3-HAA is further supported by the finding that co-cultures of $CD4^+$ T cells with DCs, in the presence of kynurenine and 3-HAA, increased the production of $Foxp3^+$ T_{reg} cells and immunosuppressive transforming growth factor-β (TGF-β) in a nuclear coactivator 7-dependent pathway.[102] NAD+, a fundamental co-enzyme involved with DNA repair, cell growth, and metabolism also has a key role in mucosal immunity and chronic inflammation. Notably, a reduction in NAD+ levels can lead to impaired phagocytosis, heightened reactive oxygen species damage, increased activation of the inflammasome and therefore result in increased susceptibility and severity of inflammation.[93] In addition to the kynurenine pathway, de novo biosynthesis of NAD+ can occur via the Preiss-Handler pathway from the precursor nicotinic acid.[103] However, it should be noted that in mammalian tissue, salvage pathways dominate the production of NAD+ by recycling derivatives of cellular metabolic reactions such as nicotinamide.[104,105]

4 | 5-HT, KYNURENINE AND GUT MICROBIOTA

4.1 | Microbiota impact on EC cells/5-HT production

In the intestinal mucosa, the close proximity between the microbiota, EC cells, and immune cells suggests the interaction between these constituents may influence the pathophysiology of intestinal inflammation.[19] Colonic EC cells express several nutrient- and metabolite-sensing GPCRs, including those that sense microbially derived short-chain fatty acids such as the free fatty acid receptor 2, and the olfactory receptors, OLFR78 and OLFR558.[106] In a recent study, Yano et al. demonstrated that select gut microbes and their metabolic products, including acetate, butyrate and propionate, can directly signal colonic ECs to promote Tph1 expression, modulate 5-HT biosynthesis, effect luminal and circulating 5-HT contents, and alter host physiology.[107] Short-chain fatty acids interacting on GPCR have also been shown to impact muscle contraction and motility within the gut via 5-HT signaling.[108]

Microbes may also directly modulate 5-HT production from EC cells via toll-like receptors (TLRs).[18] TLRs respond to bacterial ligands and are expressed on a variety of cells, including EC cells.[109] These receptors play an important role in innate immune surveillance. TLR2 in particular, has been shown to have a crucial role in 5-HT production in the gut.[110] Recently, we demonstrated that altering microbial composition with antibiotic administration greatly reduced EC cell number, colonic 5-HT levels, and TLR2 expression in C57BL/6 mice.[110] In alignment with these findings, Ge et al. illustrated that antibiotic-induced dysbiosis promoted decreased levels of 5-HT and Tph1 and slowed transit time and GI motility in C57BL/6 mice.[111] In our study, *TLR2*$^{-/-}$ mice revealed significantly decreased EC cell number and colonic 5-HT concentration in comparison with wild-type (WT) mice.[110] In a similar vein, expression of the intracellular bacterial sensor, nucleotide-binding oligomerization domain-containing protein 1 (NOD1), is modulated by 5-HT and TLR2, and has also been shown to inhibit SERT activity in Caco-2/TC7 cells.[112] Increased EC cell number and colonic 5-HT concentration have been reported in infection with the murine intestinal nematode, *Trichuris muris*.[113] It has also been shown that TLR2 plays an important role in mucosal 5-HT production in the gut by resident microbiota as well as by *T. muris*.[110]

Further evidence illustrating the pivotal role the gut microbiota plays in 5-HT production can be gleaned from germ-free (GF) studies. In GF mice, serum concentrations of 5-HT were reduced compared to conventionally raised mice. Furthermore, GF mice displayed reduced Tph1 and increased SERT mRNA in the proximal colon.[114] Similarly, Reigstad et al. demonstrated that conventionally raised mice and mice colonized with human fecal microbiota, despite unchanged EC cell number, had increased levels of both Tph1 protein and 5-HT concentration in the colon compared with their GF counterparts.[115] The authors propose that the microbial impact on host 5-HT expression is directly responsible for these changes since exogenous/luminal 5-HT was not detected in the transferred microbial samples.

4.2 | 5-HT impact on the gut microbiota

Our laboratory has recently elucidated the influence that 5-HT has on the intestinal microbiota and colitis, illustrating 5-HT's direct impact on bacterial growth in a species-dependent manner and its indirect impact on bacterial composition via antimicrobial peptides (AMPs), particularly β-defensins (Figure 2). In this study, microbial composition differed significantly in $Tph1^{+/-}$ compared with $Tph1^{-/-}$ mice. Notably, GF mice colonized with $Tph1^{-/-}$ microbiota had increased abundance of mucin degrading *Akkermansia,* a touted "next-generation probiotic". We also demonstrated that the altered microbiota compositions observed play a key role in susceptibility to colitis.[28] In addition, $SERT^{-/-}$ mice, which have increased levels of 5-HT in the intestine,[112] exhibited significant alterations in microbial composition, particularly a significant increase in Firmicutes abundance in $SERT^{-/-}$ mice compared to $SERT^{+/+}$ mice.[116]

Intriguingly, by potentially acting as efflux pump inhibitors, SSRIs have been shown to have antibiotic effects primarily with regard to Gram-positive microorganisms, including *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Campylobacter jejuni*.[117] In addition, in vitro studies have indicated that 5-HT stimulates the growth of commensal strains of *Escherichia coli*, *Enterococcus faecalis*, and *Rhodospirillum rubrum* when added to the nutrient medium. It is speculated that the stimulatory effect of 5-HT on microbial growth may be due to the presence of 5-HT receptors on the surface of these microorganisms that may also bind related compounds such as indole.[118]

The 5-HT-microbiota axis is further highlighted by the multitude of microorganisms that have intimate interactions with 5-HT biosynthesis. For instance, though the enteric pathogen, *Citrobacter rodentium*, increases 5-HT release and decreases SERT immunoreactivity ten days post-infection. It has also been touted to sense 5-HT in the gut directly via membrane-bound histidine sensor kinase, CpxA.[119] Through this kinase, 5-HT is able to decrease the expression of virulence genes in this enteric pathogen.[120] Moreover, 5-HT enhances the adherence and invasion of commensal *E. coli* strains into Caco-2 cells as well as in murine gut submucosa by amplifying signals in colonic epithelial cells.[121]

The bidirectional relationship between host and microbiota is neatly reflected in the ability of *Turicibacter sanguinis*, a spore-forming gut bacteria, to uptake 5-HT via a neurotransmitter sodium symporter-related protein structurally similar to mammalian SERT *T. sanguinis* then is able to affect host expression of a number of metabolically relevant pathways, suggesting that host-derived 5-HT not only impacts bacteria directly but these bacteria, in turn, affect the host physiology.[122]

4.3 | Gut microbes and the kynurenine pathway

Though the cause is unknown, current theories suggest that IBD is predicated on an inappropriate immune response in genetically susceptible individuals to commensal microbes and other environmental factors.[123] Thus, the influence of the kynurenine pathway in host-microbial defense is increasingly of interest in the study of IBD and intestinal inflammation.[80] Tissue damage associated with the aberrant inflammation in IBD provides points of entry for bacteria, both commensal and pathogenic, into the intestinal mucosa.[124,125] As mentioned above, increases in IDO expression and subsequent reductions in local tryptophan concentration in areas of damage may inhibit the growth of invading microbes.[125,126]

IDO's connection with the innate and adaptive immune response provides ample evidence of the role that the kynurenine pathway plays in antimicrobial defense. Activation of bacterial-sensing TLRs on innate immune cells has been reported to induce IDO1 expression.[90,94,127] Conversely, TLRs are downregulated in IDO-deficient mice.[128] Through this signaling, DCs are able to prompt immunosuppressive and anti-infectious activity via downstream inhibition of T cell survival and proliferation, and upregulation of T_{reg} activity.[2,89–91,94] Harrington et al. demonstrated that IDO-deficient mice had elevated intestinal secretions of non-specific IgA antibodies conferring increased colonization resistance in C. rodentium infection and had considerably diminished markers of C. rodentium-induced intestinal inflammation than WT mice.[129]

Gut microbes also directly degrade tryptophan, pulling the substrate away from the kynurenine and 5-HT

pathways, and impacting colitis.[2] Caspase recruitment domain-containing protein 9 (CARD9), whose associated gene is linked with IBD susceptibility, is critical for triggering appropriate inflammatory cascades in response to pathogenic microorganisms.[130] *CARD9*$^{-/-}$ mice are more susceptible to colitis and have significant alterations in the gut microbiota compared to *CARD9*$^{+/+}$ mice, notably decreases in Actinobacteria and *Lactobacillus reuteri*.[131] The transfer of this altered *CARD9*$^{-/-}$ microbiota to GF WT mice, not only had pro-inflammatory effects, but also increased WT susceptibility to colitis. Intriguingly, this increased colitis susceptibility was also associated with impaired microbial tryptophan metabolism and AhR activation via kynurenines, a pathway which promotes T_{reg} differentiation and impacts IL-22 production.[97,131] This study neatly illustrates the linkage between tryptophan metabolism, microbial influences, and host responses and the role of these factors in the context of colitis.

Gut microbial composition and function may also be modified via KYNA.[132] Pathogenic *E. coli,* a microbe often implicated in ulcerative colitis (UC), can via aspartate aminotransferase, convert kynurenine to KYNA.[133,134] Furthermore, in human myeloid DCs, *E. coli* infection has been shown to induce immunoregulatory mechanisms involving increased IDO and IL-10 expression.[135] IDO is also implicated in several extra-intestinal infections. By limiting tryptophan, IDO has been shown to suppress parasitic replication in *Toxoplasma gondii* infection[80] as well as control viral spread and replication in flavivirus[136] and retroviral infection.[137] Moreover, high IDO expression is found in the gut mucosa during the early stages of HIV infection.[138]

5 | 5-HT AND THE KYNURENINE PATHWAY IN GUT INFLAMMATION

5.1 | 5-HT and experimental colitis

In experimental colitis models, such as trinitrobenzene sulfonic acid (TNBS), dinitrobenzene sulfonic acid (DNBS), or dextran sulfate sodium (DSS), an increase in 5-HT content has been documented reinforcing similar findings in IBD patients.[139–141] Our lab has reported that mice are less susceptible to chemical- and infection-induced colitis when gut mucosal 5-HT content is significantly reduced, either by using Tph1 enzyme inhibitors or by knocking out the *Tph1* gene.[50,142] As an explanatory mechanism, we have further identified a key pro-inflammatory role of 5-HTR7 on DCs; 5-HT activates DC-expressed 5-HTR7 to initiate immune mechanisms and, thus, contributes to intestinal inflammation.[61,143] The relationship between 5-HT and gut inflammation was further supported by Gershon et al., who reported exaggeration in TNBS colitis in *SERT*$^{-/-}$ mice which have increased 5-HT in the gut.[144] Despite the above evidence, some recent studies by Spohn et al. have brought forward new insights regarding the anti-inflammatory role of 5-HT in the mucosa, specifically the protective and recuperative actions of intestinal epithelial 5-HTR4 activation in a chemical colitis model. 5-HTR4 is required for the physiological maintenance of epithelial integrity.[145] Increased epithelial proliferation, migration and resistance to oxidative stress-induced apoptosis seem to underlie the anti-inflammatory effects of 5-HTR4. Spohn et al. explained the contradictory roles of 5-HT in gut inflammation by hypothesizing that 5-HTR4-associated anti-inflammatory action is present in the basal condition whereas the 5-HTR7 pro-inflammatory effects predominate in pathological states.[6] These observations suggest that the action of mucosal 5-HT in the gut is highly dependent on the type of receptor, type of immune cells and the local environmental conditions. Further supporting this, Gershon et al. have demonstrated that in experimental colitis, the actions of mucosal and neuronal 5-HT are distinct. Interestingly, during colitis, 5-HT synthesized by Tph2 in the serotonergic neurons of the ENS was found to be anti-inflammatory and neuroprotective, whereas mucosal 5-HT was regarded as pro-inflammatory.[146]

Cytokines, immune signaling molecules, play a critical role in gut inflammation. The relationship between 5-HT and cytokines is bidirectional. Pro-inflammatory cytokines IL-1β, TNF-α, IL-6, IFN-γ, and low levels of the anti-inflammatory cytokine, IL-10, reduce the expression and function of SERT (Figure 2).[147–150] However, at higher concentrations, IL-10 increases the expression of SERT on epithelial cells via the PI3K signaling pathway.[147] During intestinal inflammation, both the Th1 cytokine, IL-1β, and the Th2 cytokine, IL-13, significantly enhance the secretion of 5-HT from EC cells via the activation of IL-1 and IL-13 receptors (Figure 2).[151,152] Moreover, IL-13 also increases the synthesis of mucosal 5-HT by upregulating Tph1 expression (Figure 2).[152,153] However, the effect of IL-13 and IL-1β on neuronal 5-HT is yet to be determined.

Recently, another member of the IL-1 family, an alarmin cytokine, IL-33, was found to activate the ST2 receptor on EC cells triggering calcium influx via a non-canonical phospholipase C-γ1- transient receptor potential ankyrin 1 signaling pathway to induce 5-HT release. It has also been hypothesized that the serotonergic neurons might release neuronal 5-HT by IL-33 activation regardless of their distinct synthetic mechanism.[154] As mentioned above, during inflammation, 5-HT itself also increases the secretion of pro-inflammatory cytokines like IL-1β, IL-6, and IFN-γ from macrophages, DCs and NK cells.

5.2 | Kynurenine and experimental colitis

The kynurenine pathway plays a complex role in controlling immunological processes in the context of experimental intestinal inflammation. As mentioned above, of the two isoforms of IDO, IDO1 seems to have a more influential role in intestinal immunoregulation and homeostatic control than its counterpart.[127] Both genetic and chemical manipulation of IDO1 have elucidated its role in colitis. For instance, in comparison to WT, *IDO1*$^{-/-}$ mice administered 1%–2% DSS for 7 days displayed a marked reduction in markers of inflammation.[128] Pharmacological manipulation of the IDO1 by the inhibitor, 1-methyl-L-tryptophan, produced similar results. Significant downregulation of TLRs and NF-κB signaling was also detected in these IDO-deficient mice.[128] It is crucial to note that under normal conditions, mice with IDO1 deficiency did not exhibit pathophysiological changes to the gut, suggesting that IDO1 predominantly acts as an immune regulator under periods of inflammatory insult or more specifically, in the case of DSS, mucosal barrier disruption.[128,155] In contrast, Ciorba and colleagues found conflicting results to the above when using the TLR9 agonist, oligodeoxynucleotide containing immunostimulatory sequences (ISS-ODN) to induce IDO1 in both epithelial and immune cells of the gut.[127] The induction of IDO1 induced a protective impact in both DSS and TNBS colitis models, and conversely, when IDO1 was inhibited, colitis severity increased.[127] Interestingly, despite the differing mechanism behind the DSS and TNBS models,[155] in a DSS model, intraperitoneal injection of ISS-ODN prior to DSS administration also lessened the severity of colitis.[127]

In alignment with Ciorba's findings, further work within the Th1-mediated TBNS models[155] suggests that deficiencies in IDO1 exacerbate intestinal inflammation. Takamatsu et al. showed that WT mice administered TNBS displayed upregulated IDO1 expression in both the lamina propria and submucosa of the colon.[156] Mice with genetically or pharmacologically induced depletion of IDO1 showed more severe colitis and decreased Foxp3$^+$ T_{reg} cells at the site of inflammation than their WT counterparts.[156,157] These effects may be attributed to diminished T_{reg} activity, suggesting a critical role of IDO1 in immunosuppression in the colon.[156] Because of IDO1's known impact on T cell survival and proliferation,[90,91] the T cell-dependent TNBS model of colitis[155] is a crucial way in which to study the immunoregulatory effects of IDO. The above findings suggest that IDO plays a critical role in the downregulation of the Th1 inflammatory response and the mucosal immune response, and is particularly adept at regulating inflammation in the TNBS model of colitis.[157]

In addition to IDO1, downstream enzymes that act within the kynurenine pathway have also demonstrated immunoregulatory roles in intestinal inflammation. KMO, the enzyme which converts kynurenine to 3-HK, and thus to some extent, controls the amount of kynurenine in the system, plays a significant role in TNBS colitis severity. *KMO*$^{-/-}$ mice, which have increased kynurenine levels and low levels of 3-HK, showed decreased colitis severity, increased Foxp3$^+$ T_{reg} cells, and increased levels of the anti-inflammatory cytokines, IL-10 and TGF-β in comparison to their WT counterparts.[158] These findings suggest an anti-inflammatory and protective role of kynurenine in TNBS induced colitis.

From the above evidence, it is clear that IDO plays a critical and complex role in experimental colitis. Evidence from TBNS models of colitis offers that IDO1 exerts its immunomodulatory effects in a T cell-dependent manner. Work in DSS, a colitis model primarily predicated on barrier disruption,[155] suggests that IDO1 has a more complicated relationship with intestinal inflammation and that context and form of inflammatory insult may play a large role in whether IDO1's immunological influence has a net positive or net negative effect on the host. Contrasting results within this model may be attributed to differences in DSS administration length and dosage, location-specific microbial differences or the mode of IDO1 manipulation (genetic or chemical). Work in DSS models also suggests that IDO may act via T cell-independent immunoregulatory mechanisms such as increasing anti-inflammatory downstream metabolites or local depletion of tryptophan.

5.3 | Clinical implications of 5-HT in GI disorders

IBD, comprised of Crohn's disease (CD) and ulcerative colitis, is a chronic relapsing inflammatory condition of the GI tract with a rising incidence worldwide.[159] In CD patients, an increase in EC cell number and 5-HT content has been documented in the intestinal mucosa, and increased mucosal 5-HT signaling has been shown to contribute to the severity of inflammation in active disease.[151,160–163] Recently, it has been reported that active CD patients have elevated Tph1 and 5-HT receptors and downregulated SERT expression in the intestinal mucosa as well as increased plasma/serum 5-HT levels.[160,164,165] However, the role of 5-HT in UC is somewhat controversial with groups reporting both increased and decreased 5-HT signaling.[35] Furthermore, changes in 5-HT signaling occur in other intestinal disorders with underlying inflammation like IBS and celiac disease.[166,167] In fact, the role of 5-HT in IBS is so well established that 5-HTR3 and 5-HTR4 targeted therapies are among approved treatments for IBS.

Studies on 5-HT-increasing SSRIs and their association with IBD have further highlighted the importance of 5-HT signaling in gut inflammation in a clinical setting. Chronic intake of SSRIs, such as sertraline increases the risk of developing microscopic colitis. Even though the mechanism is not yet known, it is postulated that SSRIs and other medications such as nonsteroidal anti-inflammatory drugs, may trigger colonic inflammation in genetically predisposed individuals or may lead to the development of more apparent GI symptoms in a previously undiagnosed patient.[168] However, other researchers have found that SSRIs can have no effect or have a beneficial/protective effect against IBD.[169–171] However, it should be noted that these studies were observational, uncontrolled and nonrandomized, and the discrepancies in these findings may be due to the small sample size and/or short follow-up periods.[171] Therefore, it is pertinent to conduct further randomized controlled trials with larger sample sizes and follow-up periods greater than 12 months to clearly understand the impact of SSRIs on gut inflammation.

5.4 | Clinical implications of kynurenine in GI disorders

In IBD, the clinical evidence of the relationship between the kynurenine pathway and intestinal inflammation has largely been investigated with regard to the impact of IDO1. Driven by IDO, the depletion of tryptophan via the kynurenine pathway and its influence on immune regulation and the inflammatory process has been shown to impact IBD and shape GI pathophysiology and inflammation severity.[124] IDO1 expression at baseline is relatively low within the gut. However, under the stress of inflammation, the expression of IDO1 is increased in both the gut epithelium and in immune cells residing in the underlying lamina propria,[124,126] a phenomenon which is no different in patients with active IBD. In fact, immunohistochemical analysis suggests that in both human and murine tissue, increased expression of IDO1 is consistently found in both experimental models of colitis and in IBD.[92,126,172] In IBD patients, local expression of IDO1 in the epithelium was significantly increased adjacent to areas of active inflammation.[11,98,124,126,173] An increased level of IDO1 in the intestinal mucosa of active UC patients was also indicative of more severe mucosal inflammation and endoscopic damage.[173] Remarkably, induction of local IDO1 expression in IBD patients may suggest a negative feedback loop, where IDO activity stimulated by activation of local APCs, encourages a tolerogenic and compensatory mechanism, suppressing the survival and differentiation of several T cells including Th1, Th2, and Th17 subsets, and promoting the activity and proliferation of T_{reg} cells (Figure 3).[173] Indeed, increased T_{reg} cells were highest in active UC patients versus active CD patients and healthy controls.[92,173] Furthermore, alterations in the activity of IDO may also affect intestinal motility in these diseases.[85]

Increased IDO expression inherently pulls tryptophan from the local environment of the gut and leads to increased kynurenine and downstream metabolites. Indeed, decreased tryptophan levels,[98,174] increased kynurenine levels in serum,[175] and local overexpression of IDO1 in colonic biopsies have been reported in IBD.[92,176,177] Furthermore, in patients with active CD, this local increased intestinal IDO1 is associated with a high serum kynurenine/tryptophan ratio compared to both healthy controls and CD patients in remission.[98] Intriguingly, this ratio reverted to normal levels in conjunction with lessening inflammation and is positively correlated with disease severity and C reactive protein levels.[98] Increased tryptophan metabolism (i.e. less tryptophan) detected in serum and tissue expression of kynurenine-associated enzymes, including IDO1, was also linked with disease severity in UC.[176] Interestingly, tryptophan depletion may be linked with depressive symptoms often associated with IBD.[98,177] In parallel with in vivo work, supernatants of colonic explant cultures of CD patients showed significant upregulation in kynurenine and an increased kynurenine/tryptophan ratio in comparison with tissue from healthy controls.[92] Intriguingly, the anti-TNF-α drug, infliximab, diminished IDO1 in immunohistochemical analysis in CD patients.[92] Again, this finding reinforces the idea that IDO1 may represent a local compensatory mechanism in inflammation in order to suppress overactive and damaging T cell activity and boost anti-inflammatory T_{reg} cells within the colon.[2,90–92] It should be noted, however, that recent work by Manzella et al., in both UC and CD patients, did not find significant differences in serum tryptophan, kynurenine, or the kynurenine/tryptophan ratio between active disease and healthy controls or in those patients in remission.[164] These contradictory findings within the clinical setting suggest that the role of IDO and tryptophan metabolites is complex and still a topic of debate within IBD research.

The downstream metabolite, KYNA, which has been shown to have immunoregulatory effects of its own,[177] was also found to be lower in periods of remission than in periods of intense inflammation in both UC and CD.[174] Ratios of KYNA and tryptophan, and levels of enzymes such as kynurinase and KMO positively correlate with increased endoscopic inflammation and histological damage in UC patients.[177] In addition, elevated KYNA/tryptophan ratios in UC patients were positively correlated with future negative outcomes such as increased incidents of surgery and hospitalization.[177]

Evidence from the above studies and those performed in animal models inextricably links the effect of IDO and

kynurenines to the inflammatory pathways involved in IBD. Elevated IDO expression, decreased tryptophan, increases in the kynurenine/tryptophan and KYNA/tryptophan ratios have been consistently reported in IBD patients. On a local scale, upregulation of this complex pathway may act as an important immunoregulatory mechanism via local tryptophan depletion, influencing survival and proliferation of several T lymphocyte subsets or, may simply be an indicator of intense inflammation.[173] That being said, IDO manipulation may present an interesting opportunity to regulate and pump the theoretical "brakes" of gut inflammation[11,98] and inhibit further exacerbation of potentially damaging T cell activity.[92] Nevertheless, IDO1 expression, serum measurements of kynurenine, KYNA and their respective ratios to tryptophan have great potential serving as surrogate biomarkers, and acting as diagnostic tools or therapeutic targets in IBD.[98,124,173,177]

6 | THERAPEUTIC TARGETING OF 5-HT AND KYNURENINE IN GI DISORDERS

6.1 | Targeting 5-HT in GI disorders

Clinically, 5-HT targeting drugs for GI diseases are currently in use. 5-HTR3 and 5-HTR4 have been studied extensively and targeted for the treatment of nausea, vomiting, diarrhea, and constipation. 5-HTR3 antagonists, like ondansetron, exert anti-emetic effects by their action on afferent vagal nerves in the gut and in the area postrema of the medulla oblongata. These antagonists are used to treat nausea and vomiting induced by chemotherapy and radiation therapy.[178,179] Further, other 5-HTR3 antagonists such as alosetron are used to treat diarrhea and abdominal discomfort in patients with diarrhea-predominant IBS (IBS-D), though the exact mechanism of action is not known.[180,181] Similarly, 5-HTR4 agonists like tegaserod alleviate constipation and pain in IBS-C, and in chronic constipation.[182] 5-HTR4 agonists are prokinetic agents that accelerate and strengthen the peristaltic reflex, and contribute to the regulation of propulsive motility.[183,184] The prokinetic effects of 5-HTR4 agonists are mediated by their action on receptors expressed on presynaptic enteric nerve fibres and neurons.[184,185] Unfortunately, older, non-selective 5-HTR4 agonists had off-target effects on hERG potassium channels, dopamine receptors, 5-HTR1 and 5-HTR2 that resulted in adverse cardiovascular effects and were subsequently removed from the market.[186] Increasing evidence suggests that targeting the mucosal epithelial cells with 5-HTR4 agonists helps to produce the desired clinical outcomes with greatly reduced adverse cardiovascular effects.[26,183]

With the functional classification of Tph isoforms and the evidence that gut-associated Tph1 generates more than 95% of the body's 5-HT, several research groups have probed the utilization of Tph inhibitors in the treatment of 5-HT-associated peripheral diseases. The Tph inhibitors LX-1031 and LX-1033 were the first drugs to enter clinical trials and showed promise in the treatment of non-constipating IBS.[187,188] Because of their inability to cross the intestinal barrier, the action of these drugs was restricted to Tph in the gut despite their nonselective nature.[14] With that being said, the only Tph inhibitor approved by the FDA is telotristat ethyl used to treat carcinoid syndrome diarrhoea by reducing peripheral 5-HT without affecting central 5-HT.[189] As discussed above, *Tph1*$^{-/-}$ mice and animals treated with Tph1 inhibitors are protected in several different colitis models.[50,61,142,190,191] Based on these promising results and the success in IBS clinical trials, it is clear that Tph inhibitors are promising candidates for the treatment of inflammatory disorders such as IBD.

6.2 | Targeting the kynurenine pathway in GI disorders

Interestingly, IDO has been implicated in the development, pathogenesis, and progression of GI cancers.[11] In colorectal tumors, increased expression of IDO, particularly expressed by the neoplastic epithelium and APCs, contributes to reduced local tryptophan concentrations and alters the kynurenine/tryptophan ratio.[124,125,192,193] Tryptophan metabolites can alter not only regulatory and effector T cell function and proliferation in the face of cancer,[97,194] but also alter apoptotic pathways,[195] stimulate tumor immune tolerance/limit surveillance,[196,197] and stimulate growth, proliferation, and progression.[11,195,198] As well, increased kynurenine aids in immune escape and enhances the metastatic potential of cancer cells.[84]

Intriguingly, in several studies utilizing tumorigenesis/carcinogenesis promoted by inflammatory models including DSS, *IDO1*$^{-/-}$ mice have been shown to have reductions in tumor number and size, and proliferation, particularly in neoplastic epithelial tissue when compared with WT counterparts. These findings suggest diminished IDO expression, and subsequent metabolites of the kynurenine pathway may have significant beneficial effects in colitis-associated cancer in vivo.[195,198,199] In vitro work in human colorectal cells by Liu et al. also demonstrated that chemical inhibition of IDO by 1-methyl-L-tryptophan suppressed proliferation, induced mitochondrial injuries, and caused apoptosis of cancer cells. These effects were also reflected in vivo, where mice subjected to azoxymethane and DSS exhibited the protective effect of 1-methyl-L-tryptophan inhibition in

inflammation-induced colon carcinogenesis.[199] However, there is recent evidence of 1-methyl-L-tryptophan having modes of action other than IDO inhibition, making it a non-specific inhibitor.[200] Thus, it is possible that the protective effect of 1-methyl-L-tryptophan in colon cancer models might be due to either IDO inhibition or other off-target effects. Evidence from several studies indicates that IDO inhibitors may, in conjunction with more traditional cancer therapies, prove beneficial in colorectal cancer treatment.[11,195,197] In fact, many small-molecule IDO1 inhibitors are being investigated in combination with immunotherapy, chemotherapy or radiotherapy in phase II and III clinical trials for different types of cancers.[201]

Alterations in the IDO expression, kynurenine/tryptophan ratios and ratios of downstream metabolites including quinolinic acid and KYNA have also been associated with IBS-D patients,[202] diverticulitis,[126] and celiac disease.[203]

7 | INTERACTIONS AND INTERDEPENDENCE BETWEEN 5-HT AND THE KYNURENINE PATHWAY

There is emerging evidence that suggests both the 5-HT and kynurenine pathways exert influence on the immune response, the gut microbiota, and gut inflammation. While the metabolites of the kynurenine pathway are largely immunosuppressive and anti-inflammatory, mucosal 5-HT leans toward a pro-inflammatory nature in pathological conditions and an anti-inflammatory state in basal conditions.[6] It appears once intestinal inflammation is triggered; both Tph1 and IDO1 dependent pathways are upregulated. However, whether their contributions to intestinal inflammation and immune regulation are interconnected is not yet clear.

Evidence of several key interactions between these pathways are discussed below and illustrated in Figure 4:

1. 5-HT can enter portions of the kynurenine pathway via conversion to 5-hydroxykynuramine by IDO1.[2] 5-HTP can also be converted to 5-hydroxykynurenine by IDO1 which subsequently can convert to 5-hydroxykynuramine.[2] In addition, two 5-methoxylated kynuramines, N1-acetyl-N2- formyl-5- methoxykynuramine and N1-acetyl-5-methoxykynuramine, major CNS-associated metabolites of melatonin, are formed both enzymatically by IDO as well as in non-enzymatic reactions. However, physiological concentrations of these 5-methoxylated kynuramines are undetectable under normal conditions.[204]
2. Li et al. have clearly demonstrated that melatonin, an end product of the 5-HT pathway, enhances the kynurenine and inhibits the 5-HT pathway. Using PC12 cells, they found that overexpression of AANAT, the enzyme involved in melatonin synthesis, inhibits IDO1 expression. Conversely, increased IDO1 impedes AANAT. These findings suggest that changes in the key enzymes of one pathway result in the alteration of key enzymes in the other. Melatonin treatment up-regulated the expression and relocation of forkhead box protein O1 to the nucleus to induce IDO1 expression, seemingly in order to maintain a balance between the 5-HT and kynurenine pathways.[13]
3. Kynuramines may be important as endogenous agonists or antagonists of 5-HT receptors. 5-hydroxykynuramine showed an affinity for 5-HTR2 and 5-HTR3 in the smooth muscle of the rat aorta, the guinea pig ileum, and the rat stomach fundus which resulted in contractile responses. Besides its effect on smooth muscle, 5-hydroxykynuramine is a potent inhibitor of 5-HT-induced platelet aggregation.[204] This may provide a means of controlling over-production of 5-HT, not only by acting as a substitute catabolic pathway of 5-HT but also by impeding its biological actions. As the influence of 5-HT and kynurenines in gut inflammation becomes more evident, manipulating this link in the metabolic pathway may provide a novel therapeutic target for inflammatory disorders with 5-HT-kynurenine imbalance.
4. Kynurenine is an endogenous ligand of AhR which activates the AhR pathway.[205] Recently, it has been shown that 5-HT can also activate the AhR pathway in intestinal epithelial cells.[36] Whether 5-HT is a direct ligand of AhR is yet to be determined, however, it is evident that both kynurenine and 5-HT activate a common AhR pathway.

8 | SUMMARY AND PERSPECTIVES

5-HT and kynurenine, the two endogenous pathways of tryptophan metabolism, are essential players in both human health and disease. These metabolites directly and indirectly influence several components of the innate and adaptive immune system, the gut microbiota, and gut inflammation. In fact, almost all cells of the immune system are profoundly regulated by 5-HT in respect to their maturation, development and function.[25] Kynurenine and its downstream metabolites are also important mediators of immune-inflammatory responses.[206] From independent

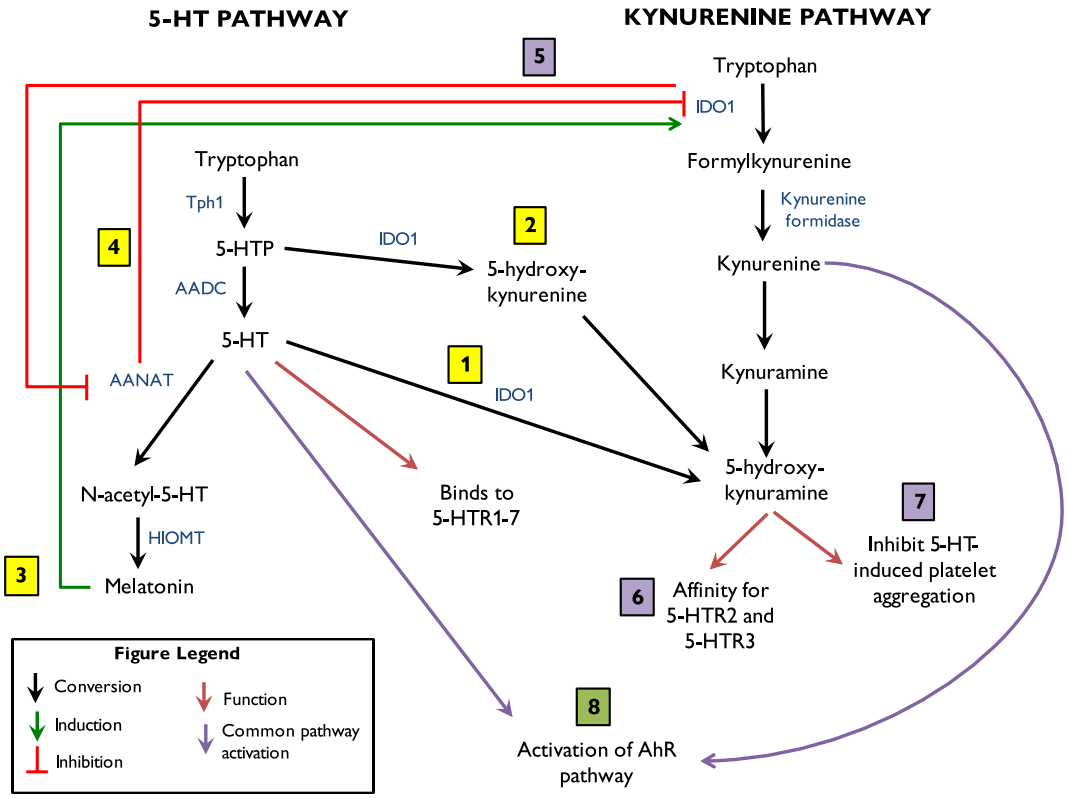

FIGURE 4 Interactions between 5-HT and kynurenine pathways of tryptophan metabolism. Effects of 5-HT pathway on kynurenine pathway (numbers in yellow): (1) 5-HT enters portions of the kynurenine pathway via conversion to 5-hydroxykynuramine by the rate-limiting enzyme, IDO1. (2) 5-HTP can also be converted to 5-hydroxykynurenine by IDO1. (3) Melatonin induces IDO1 expression. (4) Overexpression of the enzyme AANAT inhibits IDO1 expression. Effects of kynurenine pathway on 5-HT pathway (numbers in purple): (5) Increased levels of IDO1 inhibit AANAT. (6) 5-hydroxykynuramine has affinity for 5-HTR2 and -3 in smooth muscle cells. (7) 5-hydroxykynuramine inhibits 5-HT-induced platelet aggregation. Common effect (number in green): (8) Both 5-HT and kynurenine activate the AhR pathway. All the enzymes are represented in blue

pre-clinical and clinical studies, it is known that both 5-HT and kynurenine metabolism and signaling are altered during intestinal inflammation, particularly in IBD, with 5-HT mainly acting in a pro-inflammatory manner and kynurenine largely promoting anti-inflammatory effects.[25,92,158] Even though both pathways are intricately associated with gut inflammation, it remains unclear whether they interact and influence each other in relation to intestinal pathophysiology. It bears repeating that although each pathway is critical in maintaining healthy homeostasis, the two pathways of tryptophan metabolism are extremely unequal. Not much is known about how endogenous tryptophan metabolism is equilibrated and whether the human body is harmed when the balance of the 5-HT-kynurenine pathways is disturbed.

As illustrated in Figure 4, it is clear that some components of the 5-HT pathway are either converted to or upregulate critical constituents of the kynurenine pathway. The study of the pathophysiology of depression has brought forward the hypothesis that during depression, activated IDO metabolizes tryptophan into kynurenine and shifts the balance from the 5-HT to the kynurenine pathway. Thus, activation of this pathway causes a shortage in 5-HT production.[1] Whether this hypothesis is relevant in inflammatory disorders is yet to be determined. It is tempting to speculate that in IBD, enhanced immunosuppressive kynurenine and its downstream metabolites may inhibit the pro-inflammatory hormone, 5-HT, in an attempt to control the pathological inflammation. It is also conceivable that an increase in mucosal 5-HT under inflammatory conditions may reduce the availability of tryptophan destined for the kynurenine pathway. In fact, during the study of functional GI disorders, it has been seen that changes in intestinal lipid metabolism disrupt the integrity of the enteric nervous system and alter the 5-HT-kynurenine balance in intestinal tissue. Specifically, oxidized phospholipids caused a marked reduction in the intestinal 5-HT level, shifting tryptophan metabolism toward kynurenine production.[207] Thus, it is of utmost importance that both the kynurenine and 5-HT pathways are studied collectively to gain a complete and thorough understanding of the involvement and alterations of endogenous tryptophan metabolism in gut inflammatory disorders.

Another avenue to consider is the role of gut microbiota in 5-HT-kynurenine interactions. As key sensory transducers in the gut, it is not surprising that the impact of the microbiota and its metabolites on EC cells and the subsequent effects on 5-HT production are emerging as a major factor in gut physiology and the pathogenesis of IBD. The gut microbiota also regulates the kynurenine pathway metabolism.[208] Notably, 4%–6% of ingested tryptophan is directly metabolized by the intestinal microbiota.[2,35] Thus, tryptophan metabolism is directly and indirectly controlled by intestinal microbes. It, therefore, seems feasible that the delicate balance between the 5-HT and kynurenine pathways in health and disease might, in fact, be regulated by the gut microbiota. The importance of the 5-HT and the kynurenine pathways is further underscored by the study of molecules targeting the rate-limiting enzymes, Tph1 and IDO1, in clinical trials of IBD and different types of cancer. However, the complex tripartite relationship of 5-HT, kynurenine, and microbial interactions demands further investigation in the context of gut inflammation and may, in the future, provide promising targets for therapeutic intervention.

9 | CONCLUSION AND KEY TAKEAWAYS

In conclusion, tryptophan-derived 5-HT and kynurenines play critical roles in immune activation, microbiota regulation, and intestinal inflammation. However, much is still unknown regarding the importance of the 5-HT-kynurenine balance and the interactions of these metabolites during different gut pathologies. Thus, these pathways prove an important area for future GI research. Though the above discussion is extensive, the key takeaway messages are as follows:

- Tryptophan metabolism occurs mainly through the kynurenine and, to a minor extent, the 5-HT pathways in the gut. The vast majority of the body's 5-HT is produced in the gut by EC cells.
- These metabolites have essential roles in regulating immune cell function, intestinal inflammation, as well as altering the production and suppression of inflammatory cytokines.
- Both 5-HT and kynurenine have a considerable impact on the gut microbiota. The gut microbiota can also influence the production of 5-HT and kynurenine.
- Complex interactions exist between the two pathways to maintain gut homeostasis and shape gut physiology.
- Clinically, alterations in both the 5-HT and the kynurenine pathways (particularly with regard to IDO1 expression and altered metabolite ratios) have been associated with GI disorders including IBD. Because of this, the constituents of these pathways have great clinical potential by serving as surrogate biomarkers and/or by acting as diagnostic tools or therapeutic targets in IBD.

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DISCLOSURES

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Sabah Haq and Jensine A. Grondin equally reviewed the literature and wrote the manuscript; Sabah Haq conceived the idea for the article; Sabah Haq and Jensine A. Grondin designed and created the figures; Waliul I. Khan and Jensine A. Grondin edited and revised the manuscript; Waliul I. Khan supervised the project; all authors provided critical feedback and shaped the final manuscript.

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