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Indole Derivatives, Microbiome and Graft Versus Host Disease

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Abstract

Graft versus host disease is a life-threatening complication following allogeneic hematopoietic stem cell transplantation driven by donor T cells reacting against disparate host antigens. Immune homeostasis within the gut plays a major role in the graft versus host response. Gut microbiota and its metabolites impact gut integrity, inflammation and immune activation within the gut. This review will focus on the role of indoles, a product of microbiota metabolism, on gut homeostasis and our current understanding on how that modulates graft versus host disease.

Keywords

Graft versus host disease; gut microbiome; gut metabolome

Introduction

Graft versus host disease (GvHD) is one of the primary barriers to successful allogeneic hematopoietic cell transplantation (HCT). GvHD is a complex inflammatory process that occurs when donor lymphocytes contained within the graft respond to antigens on host cells. The initial step in GvHD pathophysiology is activation of antigen-presenting cells (APCs), induced by host tissue damage from the HCT conditioning regimen [1]. Donor T cells activate in response to APCs, with subsequent proliferation and target tissue destruction. Immunosuppressive regimens aim to limit GvHD while maintaining reconstitution of the

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
immune system with donor-derived cells and the benefit of graft-versus-tumor effect. Approximately 30–50% of HCT recipients develop GvHD [2].

The role of the gut microbiome in inciting this inflammatory response was recognized in research from the 1970s, where germ-free mice were relatively protected from GvHD in murine HCT models [3]. However, it was not until high throughput sequencing methods evolved to better describe the composition and relative abundance of bacterial species that deeper understanding of the microbiome, dysbiosis, and the interaction of the gut microbiome with the host immune system became more apparent (reviewed in [4]).

Over the past decade, advances have been made in our understanding of the gut microbiome impact on mucosal integrity, the crosstalk between the microbiota and the immune system within the gut, the alterations in microbiome resulting from the HCT intervention, and their deleterious impact on HCT outcomes. More recently, a deeper understanding of the metabolome, the bioproduct of the microbiota and its interaction with the host epithelium, have shed light on how microbial metabolites may impact immune activity and GvHD involving the gut.

In this review, we summarize recent understandings of the association of microbial metabolites with GvHD with a focus on indoles, a key metabolome with manifold signaling properties in the gut that modulates mucosal immunity and the GvHD response.

Maintaining immune system homeostasis within the gastrointestinal tract

Immune homeostasis within the gastrointestinal (GI) tract is the result of complex interactions between APCs, innate and adaptive immune cells, gut epithelium, microbiota and their metabolites.

APCs, Dendritic cells (DCs) and Macrophages, within the lamina propria have a protective role against bacterial pathogens. Macrophages phagocytose microorganisms that penetrate the gut epithelium and DCs sense the antigenic and metabolic contents of the gut lumen and subsequently traffic to the secondary lymphoid tissue and activate immune responses. Macrophages produce IL-1b, which supports Th17 cell differentiation within intestinal tissue. This is potentiated by microbiota signals, such as the interaction with segmented filamentous bacteria and intestinal epithelial cells, that lead to Th17 differentiation.[5] Activation of Th17 cells offers protection from GI pathogens, and is balanced by activation of regulatory T cells (Tregs), which confer tolerance towards commensal bacteria during early development [6,7].

Innate lymphoid cells (ILCs) are present within the gut mucosa and are classified into 3 types based on their cytokine response and the pathogens they respond to. ILC3s generate IL-17a and IL-22 in response to enteric bacteria.[8] Specifically of interest to this review, ILC3s signal through the aryl hydrocarbon receptor (AHR) and the Retinoic acid receptor-related orphan receptor-γt (RORγt)+ and play an important role in maintaining the epithelial barrier function. They support intestinal stem cells (ISCs) within the intestinal crypts through the secretion of IL22, which also induces Reg3α production by Paneth cells. Models indicate that IL-22 could reduce intestinal GvHD pathology by enhancing secretion
of peptide defensins from gut epithelial cells and promoting the survival of ISCs [9,10]. Reg3α is an antimicrobial peptide that is concentrated in the mucus layer and protects intestinal epithelial cells from potential pathogens present within the intestinal lumen. In murine GvHD, it prevents apoptosis of ISCs and Paneth cells [11]. More recently, the protective impact of glucagon-like-peptide-2 on Paneth cells and ISCs was shown in murine models to decrease acute GVHD [12]. In patients undergoing HCT, Paneth cell count within duodenal biopsies, and serum Reg3α levels (following destruction of Paneth cells), are closely linked to the severity of GvHD [13,14]. The details of these interactions have been comprehensively reviewed recently [15].

Microbiota and Metabolic alterations in the gut during HCT

Loss of intestinal microbiome diversity is associated with increased transplant-related mortality (TRM) [16]. Acute GvHD is associated with expansion of Enterobacteriales, Lactobacillales, Proteobacteria and Akkermansia, with loss of Clostridia [16,17]. Among cases with favorable outcome, on the other hand, Clostridiales mostly of the genus Blautia dominate, suggesting an imbalance between protective and pathogenic bacteria as a contributing factor to intestinal inflammation [18] [19]. In murine GvHD models, administration of probiotic Lactobacillus species in one study [16], and a mixture of 17 species of Clostridia in another study [20], successfully abrogated development of GvHD.

The impact of microbiome alterations on GvHD is in part related to changes in intestinal metabolites, resulting from bacterial metabolites of ingested food or host-microbial cometabolism [21]. Examples of such alterations are the observations of decreased intestinal short-chain fatty acids (SCFAs) in murine GvHD. The SCFA Butyrate is a main energy source for colonic epithelial cells, and is generated by gram-positive anaerobic bacteria from complex carbohydrates [22]. SCFA have anti-inflammatory effects by inhibiting NF-κB signaling and reduce oxidative DNA damage and support self-renewal of colonic epithelial cells from intestinal stem cells. Butyrate upregulates expression of tight junction proteins and production of mucin by goblet cells [20]. In the gut, SCFAs bind G-protein coupled receptor 43 (GPR43), which mediates activation of NLRP3 inflammasome in IECs, resulting in IEC protection and amelioration of GVHD damage [23].

In patients with GvHD, fecal Butyrate is decreased among patients with gastrointestinal GvHD [24]. Enterococcus expansion, associated with acute GVHD and blood stream infection, is in part dependent on lactose utilization. In murine models, lactose depletion inhibits enterococcal expansion and subsequent GVHD, and in HCT recipients, patients with lactose malabsorption alleles have prolonged enterococcus domination [25]. Bile acids produced by the liver are metabolized by enteric bacteria into secondary bile acids. In mice, disrupted bile acid signaling compromises the gut barrier and increases bacterial translocation. Bile acids can inhibit macrophage production of inflammatory cytokines through NF-κB dependent pathways and through inhibition of NLRP3-dependent inflammasomes [26]. The NLRP3 inflammasome in host hematopoietic APCs can enhance GVHD [27], which is in contrast to its role in non-hematopoietic host tissue, as evidenced by its protective effect on IECs in the SCFA experiments. The secondary bile acid 3β-hydroxydeoxycholic acid can drive the differentiation of Tregs within the gut by acting on
These findings, in addition to the observation of significant alterations in the bile acid metabolic profile in patients with GVHD, suggest a link to these metabolites [29]. The differential effects of SCFA on the gut epithelium versus T cells has not been fully explored. The mechanisms through which bile acids, and others such as Polyamines, are involved in GvHD also remain to be fully elucidated [30].

AHR ligands, or indoles, have recently emerged as important factors in modulating immune responses within the gut, including in the pathogenesis of GvHD. AHR ligands have been shown to module both innate immunity, through AHR signaling of ILC3s and IL22 production, and adaptive immunity, through modulating TH17 cells [31]. Depletion of AHR ligands is associated with loss of ILC3 and Th17 in the gut, leading to increased susceptibility to gut infection [32]. In contrast to the beneficial effect of SCFA, the enteral administration of choline or its metabolite Trimethylamine-N-oxide (TMOA) caused increased GvHD in mouse HCT models [33]. The pro-inflammatory mechanism of TMOA was due to NF-κB translocation and activation of the NLRP3 inflammasome in macrophages leading to cleavage of caspase-1 and IL-1β secretion. In these studies, enteral administration of choline or TMOA did not significantly change the composition of the gut microbiome but did result in a series of immunological effects leading to GvHD, indicating that alterations in diet may have direct effects on host immune cells as well as secondary effects through changing the gut microbiome.

Graft versus leukemia (GvL) immune mediated clearance of leukemia cells is a beneficial effect of HCT in leukemia control and relapse prevention. The gut microbiome interactions with the immune system could impact this systemic immune response. In one retrospective study in patients undergoing HCT, microbiome composition, and specifically the abundance of Eubacterium limosum, was associated with decreased relapse rates. This impact was strongest in T cell replete grafts [34]. It is possible that the ability of this bacterium to produce SCFAs could enhance GvL activity of donor T cells and thus protect from relapse. Microbiome impact on cancer immunotherapy is also studied outside of HCT. In a trial of programmed cell death-1 inhibitors in solid tumors, patients with higher fecal SCFAs had a longer progression free survival, again pointing to a potential link between the gut microbiome, T cell activation, and tumor immunosurveillance [35].

**Indole Derivatives and their impact on gut mucosa and immunity**

Indoles represent another class of small signaling molecules derived from plant food, or endogenously produced by bacteria that metabolize tryptophan [36]. The gut microbiome plays a role in the three major tryptophan metabolism pathways (serotonin, kynurenine and AHR/indole derivatives), all implicated in a wide spectrum of human diseases [37]. Indoles have critical roles in intracellular signaling and control of cell growth across multiple taxonomic Kingdoms. In plants indoles control growth and elongation of cells in response to light [38]. Indoles synthesized by more than 85 different bacterial species inhibit bacterial growth and the invasive properties of some pathogens [39]. The structure of indoles consists of the side chain of tryptophan: a benzene ring fused to a five-membered nitrogen containing pyrrole ring [40]. Tryptophan metabolites have pleiotropic roles and serve as the chemical basis for neurotransmitters and melatonin in animals, but mammalian cells cannot synthesize...
indoles directly from tryptophan. Instead, indoles generated from tryptophan by the gut microbiota act as intra-species signaling molecules in the gut that maintain the gut epithelial barrier via activation of the AHR [41].

Homeostatic maintenance of the gut by indoles signaling through the AHR in ILCs is achieved by promoting the generation of mucin-producing goblet cells [42]. Mucin produced by goblet cells in the gut epithelium helps separate bacteria in the fecal stream from direct contact with gut epithelial cells, reducing transepithelial movement of commensal and pathogenic bacteria and associated inflammation triggered by bacteria that migrate across the epithelial basement membrane [43]. Indoles induce synthesis of proteins associated with epithelial tight junction and down-regulate markers of inflammation [44,45]. Enteral administration of indole carboxaldehyde or enhanced production of indoles by gut microbiota leads to increased proliferation of epithelial cells in the gut crypt and higher numbers of goblet cells in crypts of mice, an effect that is lost in IL10 knock-out but not in IL22 knock-out or IFNaR1 knock-out mice [42]. Treatment with IC3-carbinol increased goblet cells in the colon of mice and mucin production by gut-stem cell-derived organoids [46]. These data support a role for indoles produced by commensal enteric bacteria in maintaining the barrier function of gut epithelium and suggest that an indole-rich diet or enhanced colonization by indole-producing bacteria prior to HCT could confer protection from subsequent GvHD. Indeed, feeding mice a diet containing 10% broccoli for a week protected mice from chemically induced colitis [47].

Endogenous indole compounds have an important role in gut immune homeostasis. AHR is a key regulator of indole 2,3-dioxygenase 1 and 2 expression in DCs [48,49]. Activation of indole 2,3-dioxygenase (IDO), the initial rate-limiting enzyme of tryptophan catabolism along the kynurenine pathway, produces potent inhibitors of T cell activation and induces T cell apoptosis [50]. Its role in GvHD has been demonstrated in experimental models, with accelerated GvHD observed in IDO−/− mice [51,52]. IDO−/− donor plasmacytoid DCs have also been shown to induce more severe GvHD in mice [53]. Indole metabolites were shown to offer mucosal protection from inflammation and to limit colitis associated with infections such as Citrobacter rodentium and Candida albicans [54,55].

A variety of commensal bacteria such as Fusobacterium, Escherichia coli, Bacteroides, and Enterococcus faecalis use tryptophanase to convert tryptophan into indole and its derivatives including indole-3-carboxaldehyde (ICA) [56]. The tryptophan metabolite compounds can be absorbed by the host, and measured in the blood, tissues and urine [44,57]. Mice treated with whole body radiation develop dysbiosis with subsequent decreased indole levels in the blood [58,59].

Finally, various T cell subsets express AHR, with high expression in TH17, FOXP3+ Tregs and T regulatory type 1 cells (Tr1 cells). AHR impacts early differentiation of Th17 cells, and drives conversion of Th17 cells into anti-inflammatory Tr1 cells [60]. Treatment with AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin ameliorates colitis in a chemically induced colitis model, with increase in differentiation of Tregs and inhibition of Th17 cells through epigenetic regulation. This impact was abrogated in AHR knock-out mice [61]. AHR activation by the indole compound 1’H-indole-3’-carbonyl-thiazole-4-carboxylic acid methyl
ester resulted in increased Tregs and protected against T cell-driven colitis in humanized mice [62].

Thus AHR ligands impact various aspects of gut immunity and barrier function through effects on ILCs, DCs, T cell subsets, and goblet cells, all instrumental in the pathophysiology of GvHD.

**Indole derivatives and GvHD**

There is mounting evidence that indoles can have a major impact on early development of GvHD (Figure 1). In experimental GvHD models, mice supplemented with ICA had a dose dependent protection from GvHD, and decreased intestinal damage following radiation exposure. [63] In these experiments, ICA treatment did not change AHR signaling or IL-22 production. ICA administration did improve gut epithelial barrier integrity and decrease bacterial translocation, two critical steps in the development of GvHD, via IFN1 signaling.

Colonization of mice with tryptophanase-producing E coli protected them from radiation-induced gut injury and prolonged their survival following non-myeloablative doses of radiation compared with mice colonized with tryptophanase-deficient E. coli. Exogenous administration of ICA by daily gavage protected mice from gut GvHD and significantly improved their survival while not significantly affecting donor T cell accumulation in the portal tract and liver GvHD [63]. Notably, treatment of leukemia-bearing mice with ICA did not abrogate the GvL effect of donor T cells, indicating that enteral administration of ICA did not lead to pan-immunosuppression [63]. Thus, the capacity to dissociate GvHD from GvL with enteral administration of ICA or by colonization of the intestinal tract with tryptophanase-producing microbes may prove to be an observation that is clinically translatable with the potential for significant benefit to patients.

The clinical significance of the indole pathway in regulating GvHD and reducing TRM is supported by observational studies that have shown that levels of urinary indole metabolites are correlated with the incidence and severity of GvHD and TRM [64]. Dietary L-tryptophan is converted by tryptophanase to indole, which is absorbed and metabolized by the liver into 3-indoxyl sulfate (3-IS) and excreted in the urine [65]. Urinary 3-IS have been shown to be a good marker of gut microbiome disruption; members of the families of Lachnospiraceae and Ruminococcaceae of the class of Clostridia were associated with high urinary 3-IS levels, whereas members of the class Bacilli were associated with low levels. Low 3-IS levels within the first 10 days after HCT were associated with higher TRM and more gut GvHD [64]. The same study showed a deleterious effect of early use of systemic broad-spectrum antibiotics on 3-IS levels. In another study, tryptophan metabolites, including 3-IS, indoleacetate, indoleacetylglutamine, were decreased in patients who developed GvHD [29]. In contrast, fecal transplantation following HCT was associated with increased 3-IS in urine, revealing an improvement in microbiota diversity [66]. Thus metabolites produced by the gut microbiome may be used as surrogate markers for low microbial diversity and to identify particular microbial taxa that produce more indoles [22].
Concluding remarks and future directions

Indoles play a critical role in maintaining immune homeostasis within the gut. Murine experiments show a protective effect against radiation injury and GvHD damage. Experimental GvHD data in mice have shown that supplementing with enteral indole bypasses the need for gut colonization by commensal bacteria that make endogenous indole. In HCT recipients, many of the desirable effects of commensal bacteria are mediated through tryptophan metabolism, and urinary 3-IS level reflects microbiome diversity and correlates with HCT outcomes.

The current composition of the neutropenic diet generally relies on cooked food products while restricting fresh fruits and vegetables [67]. Indoles and more complex compounds that are degraded in the gut into indoles represent a nutraceutical present in a variety of common foods, particularly in cruciferous vegetables [68]. Data support its evaluation as a therapeutic for GvHD, with the aim to restore gut metabolomics profile to one associated with the presence of protective commensal bacterial species. Thus an approach with either the introduction of a high indole diet, with dietary servings as the study intervention, or more precisely, a supplement with a specific purified, clinical-grade indole compound, such as ICA, can be applied in a clinical trial, to test whether indole supplementation translates into better protection from gut injury and inflammation and GvHD, with improved HCT outcomes.

References


### Highlights

- Microbiota and their metabolites maintain immune homeostasis within the gut.
- Aryl Hydrocarbon Receptor ligands, or indoles, modulate innate and adaptive immunity.
- Indoles improve gut barrier integrity and protect against graft versus host disease.
Figure 1. Effects of Indoles on early GvHD pathophysiology

The initial step in GvHD pathophysiology is activation of antigen-presenting cells (APCs), induced by host tissue damage from the HCT conditioning regimen, and release of inflammatory cytokines. Donor T cells activate in response to the APCs, with subsequent proliferation, and further destruction of the gut epithelial barrier. Translocation of bacteria through the leaky epithelial barrier results in activation of macrophages and release of inflammatory cytokines, further activating T cells. In the presence of indole metabolites (produced through breakdown of tryptophan by enteric bacteria), gut epithelia integrity is maintained, through AHR signaling and ILC3 production of IL22, and through plasmacytoid...
dendritic cell signaling – resulting in expansion of Tregs, generation of IL10, and increased numbers of mucin-producing