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INVITED REVIEW

Gut Microbiome and Pulmonary Arterial Hypertension – A Novel and Evolving Paradigm

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Summary

Pulmonary arterial hypertension is characterized by perivascular and systemic inflammation. The gut microbiome influences the host immune system. Here we review the emerging preclinical and clinical evidence that strongly suggests that alterations in the gut microbiome may either initiate or facilitate progression of established pulmonary arterial hypertension by modifying the systemic immune responses. We also briefly review the relationship between the gut microbiome and preeclampsia, a vascular disease also characterized by inflammation.

Key words

Dysbiosis • Right ventricle • Inflammation

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Introduction

Pulmonary arterial hypertension (PAH) is characterized by remodeling of the pulmonary vasculature causing reduced pulmonary arterial compliance (PAC) and increased pulmonary vascular resistance (PVR), ultimately resulting in right ventricular failure and death [1]. PAH remains as a fatal disease with a median survival of only 5-7 years and an in-hospital mortality of 6 % [2,3]. Current therapies are predominantly pulmonary vasodilators, which increase exercise capacity modestly and reduce hospital admission but are expensive and not curative [1]. Sotatercept, a novel fusion protein that traps activin and growth differentiation factors involved in the proliferation of pulmonary arterial smooth muscle cells in PAH, was recently approved by the Food and Drug Administration [4]. It is the only approved antiproliferative therapy for the treatment of PAH. Sotatercept also only improves exercise capacity and reduces time to clinical worsening. It is not curative and its effect on survival is unknown. Thus, there is an unmet need for better therapies for PAH to improve survival.

Inflammation and PAH

Inflammation plays a pivotal role in the pathogenesis of PAH [5]. PAC decreases early in the pathogenesis of PAH before there is an increase in the PVR [6]. This loss of vascular compliance correlates with extracellular matrix remodeling and fibrosis in the pulmonary vessels, which is linked to chronic perivascular inflammation and immune dysregulation [7]. Clinical and experimental evidence supports the role of inflammation in PAH [5]. Abnormalities in both adaptive and innate immunity occur in PAH. Clinically, PAH is associated with autoimmune diseases, including scleroderma and systemic lupus erythematosus, and infectious diseases, such as human immunodeficiency virus and schistosomiasis infection [1]. There is perivascular accumulation of inflammatory cells including macrophages, T cells, and B cells in the pulmonary arteries in PAH patients[5]. Increased serum cytokine levels in patients with PAH are associated with

increased mortality and reduced right ventricular function [8, 9]. Patients with PAH have increased serum autoantibody levels such as antinuclear antibodies [5]. The lower number of circulating Natural Killer cells is associated with poor survival in PAH patients, suggesting that they have a protective role [10].

Several preclinical studies prove a cause-and-effect relationship between inflammation and PAH. PAH can be induced experimentally in animals by exposure to various immune stimuli, including human immunodeficiency virus, schistosomiasis, and interleukin (IL)-6 overexpression [5]. Depletion of inflammatory macrophages in chronic hypoxic calves and rats prevents remodeling of the pulmonary vascular extracellular matrix and pulmonary hypertension (PH) [5]. Athymic rats deficient in T-cells and B-cells develop pulmonary vascular disease in response to Sugen and hypoxia [11]. Immune reconstitution of the athymic rats with regulatory T cells (Tregs) reduces the severity of PH caused by Sugen and hypoxia [11]. An imbalance of CD4 helper T-cell subsets occurs in PAH lungs. While there is an increase in TH1, TH2, and TH17 CD4 helper T-cells that induce inflammatory responses, Tregs, which have anti-inflammatory effects, are decreased [11]. Finally, autoantibodies from monocrotaline rats cause PH in naïve rats, supporting the importance of abnormal B-cells in the pathogenesis of PAH [5].

Gut microbiome and immune dysregulation

The gut microbiome consists of trillions of bacteria, viruses, and fungi. The individual gut microbiome composition and functionality are determined by geographic factors, drugs, diet, exercise, and genetics [12-15]. The gut microbiome regulates many physiological functions in the host including immune regulation, the intestinal mucosal barrier, energy homeostasis, xenobiotic metabolism, vitamin synthesis and degradation, and neurological development amongst others [16]. Detrimental changes in the intestinal microbial community structure and function is called dysbiosis, which can lead to immune dysregulation and chronic systemic inflammation [17]. The gut microbiome influences the host immune system through several different mechanisms. Increased bacterial translocation and release of endotoxins, due to dysbiosis, trigger activation of macrophages through upregulation of Toll-like receptor 4 (TLR4) and T cells including TH1, TH2, and TH17 CD4 helper T cells [18-21]. In addition, the gut microbiome can impact the host immune system through release of bacterial metabolites. Some of these metabolites such as short chain fatty acids (SCFA-acetate, butyrate, and propionate) have a beneficial, anti-inflammatory effect, including activation of Treg cells through G-protein-coupled receptors (GPCR), and through epigenetic modifications induced by inhibiting histone deacetylase [17,20,21]. Alternatively, some metabolites like Trimethylamine N-oxide (TMAO) can have a proinflammatory effect through activation of macrophages and T cells [22]. Thus, either an increase in the pathogenic bacteria, or a decrease in beneficial bacteria, in the gut can dysregulate the immune homeostasis, leading to chronic systemic inflammation. Gut dysbiosis is linked to the immunopathogenesis of numerous chronic inflammatory diseases including atherosclerotic vascular disease, obesity, diabetes mellitus (both type 1 and 2), depression, alcoholic and nonalcoholic steatohepatitis, multiple sclerosis, and chronic lung allograft rejection among others [17].

Gut microbiome and PAH (A Gut-Lung Axis)

There are several compelling and evolving observations that suggest that the inflammation in PAH is initiated by intestinal dysbiosis and altered circulating microbial products (Fig. 1).

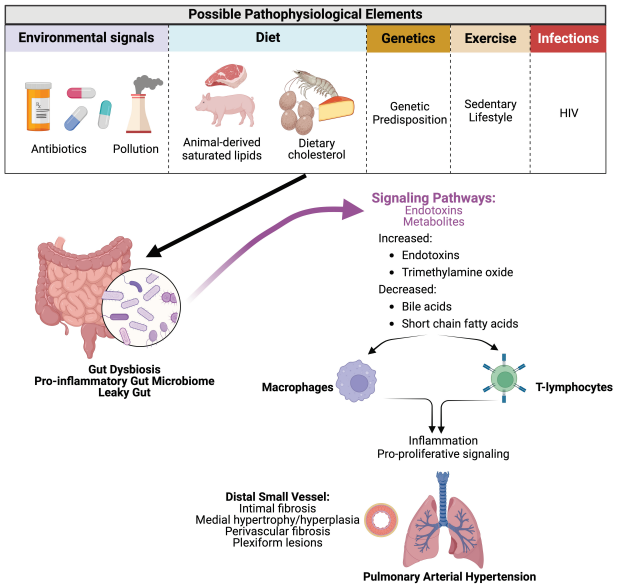

Fig. 1. Gut dysbiosis initiates or facilitates progression of pulmonary arterial hypertension by modifying systemic immune responses.

We and others have recently shown that PAH patients have a proinflammatory gut microbiome and altered circulating microbial metabolites. Compared to healthy controls, PAH patients had a less diverse and a more proinflammatory gut microbiome with reduced relative abundance of beneficial anti-inflammatory taxa and increased relative abundance of pro-inflammatory taxa, and lower plasma levels of anti-inflammatory microbial metabolites (SCFAs and secondary bile acids) and higher plasma levels of pro-inflammatory microbial metabolites such as TMAO [23,24]. Consistent with the circulating microbial metabolite levels, PAH patients have fewer copies of some of the gut microbial genes that produce anti-inflammatory microbial metabolites and a lower relative abundance of some of the species encoding these genes. PAH patients also have enrichment of species with microbial genes that encode the proinflammatory microbial metabolite trimethylamine [23,24]. Moreover, serum TMAO levels in PAH patients are associated with disease severity and poor prognosis [25,26].

Bacteriophages or phages are viruses that infect bacteria. By infecting the gut bacteria, phages can increase or decrease the abundance of the host bacteria or alter the functionality of the host bacteria without a change in their relative abundance. Thus, phages can indirectly be linked to chronic systemic diseases through their interaction with the host gut bacteria. In addition to abnormal gut bacteria, PAH patients also have altered gut bacteriophages with enrichment of *Enterococcal*, and relative depletion of *Lactococcal* phages compared to healthy subjects [24]. The increase in *Enterococcus* phage in PAH is associated with increased relative abundance of the pro-inflammatory *Enterococcus* taxa in PAH patients [24].

While human association data does not prove a direct cause-and-effect relationship between PAH and gut dysbiosis, preclinical data suggest a mechanistic role for gut dysbiosis in PAH. Preclinical animal models of PH exhibit gut dysbiosis. Wistar rats treated with Sugen and chronic hypoxia to cause PH demonstrate gut dysbiosis on taxonomy-based analysis with increased Firmicutes and decreased Bacteroidota [27]. The normal Firmicutes/Bacteroidetes (F/B) ratio helps to maintain intestinal homeostasis. Thus, altered F/B ratio, both increased and decreased, is considered as a marker of gut dysbiosis. In this animal model, serum acetate (an anti-inflammatory SCFA) levels are lower. Interestingly, gut microbiota modification with antibiotic treatment significantly suppresses the vascular remodeling, right ventricular hypertrophy, and increase in the right ventricular systolic pressure in Sugen/hypoxia rats, suggesting a causative role for gut dysbiosis in the pathogenesis of PAH [28].

Similarly, alteration of the circulating microbial metabolites either prevents or regresses pulmonary vascular remodeling in preclinical animal models. In an hypoxic rat model of PH, administration of butyrate, an anti-inflammatory SCFA predominantly derived from the gut microbiome, prevents and regresses hypoxia-induced pulmonary vascular remodeling, increase in right ventricular systolic pressure, and right ventricular hypertrophy [29]. Mechanistically, butyrate reduced infiltration of alveolar and interstitial macrophages in the lungs [29]. Parallel to this, reduction in circulating levels of TMAO, a proinflammatory bacterial metabolite, reduces right ventricular hypertrophy and pulmonary vascular remodeling in the monocrotaline-induced rat model of PH and the hypoxia-induced mouse model of PH through decreasing macrophage production of cytokines and chemokines [30].

There are also several compelling circumferential observations that suggest that the immune dysregulation and perivascular inflammation in PAH is initiated by intestinal dysbiosis and a greater burden of the circulating microbiome and microbial products. First, the pulmonary vascular macrophages encounter a greater burden of microbial products from the gut in experimental PH and in patients with liver disease and portopulmonary hypertension [31]. Serum endotoxin levels are elevated in both experimental PH and human PAH. Monocrotaline-induced PH rats have increased gut permeability, increased circulating levels of endotoxin in the portal vein, and increased circulating levels of soluble CD14, a marker of macrophage activation, in the systemic venous blood [32]. The increase in serum endotoxin levels is associated with upregulation of TLR4, the main receptor for endotoxin, in pulmonary arterial smooth muscle.

Common bile duct ligation (CBDL) in a rat recapitulates human pulmonary vascular disease related to cirrhosis [31]. Our prior work described an increased medial thickness and loss of lumen in the resistance pulmonary arteries in CBDL rats compared with sham animals [31]. In this experimental model, circulating endotoxin levels are elevated, which in turn recruit and activate pulmonary intravascular macrophages. Endotoxin released from the gut bypasses the liver

through the portosystemic shunts resulting from cirrhosis and portal hypertension, avoiding hepatic uptake and inactivation, passes through the right heart, and enters the pulmonary vasculature. In the lungs the endotoxin activates macrophages, leading to pulmonary arteriovenous malformations, capillary dilatation, and proliferative arteriopathy of the distal pulmonary arteries. Treatment of CBDL rats with the antibiotic norfloxacin decreases pulmonary intravascular macrophage accumulation and reduces pulmonary vascular remodeling [33]. Likewise, depletion of the pulmonary intravascular macrophages in the CBDL rats prevents, as well as reverses, the pulmonary vascular changes [31].

This pathological mechanism has also been described in PH associated with congenital porto-systemic shunts in the Abernathy malformation. Patients with the Abernethy malformation often develop pulmonary vascular disease, including portopulmonary hypertension and hepatopulmonary syndrome [34]. Increased systemic endotoxin levels are described in PAH associated with the Abernethy malformation [35]. Importantly, correction of the portosystemic shunt in patients with Abernethy malformation reverses PAH, suggesting a causative role for the gut-lung axis in the pathogenesis of PAH [35]. Likewise, patients with cirrhosis, who undergo a trans-jugular intrahepatic portosystemic shunt, are more likely to develop portopulmonary hypertension [36]. Patients with idiopathic PAH and heritable PAH have increased serum levels of endotoxin and soluble CD14 compared with healthy controls [32]. In patients with PAH, the increase in serum CD14 levels parallels the increase in serum endotoxin levels. Furthermore, patients with PAH have increased blood TLR4 expression compared with healthy controls [37]. Mutations in the bone morphogenic protein receptor II (BMPR II) signaling pathway underlie 80 % of heritable PAH but disease penetrance is only 20 %, suggesting a requirement for additional triggers [38]. Chronic administration of lipopolysaccharide causes PH in Bmpr2+/- mice but not in littermate controls, signifying that endotoxin-induced inflammation can be an important cofactor for disease penetrance [39]. Interestingly, a recent bidirectional Mendelian randomization study demonstrates a causal relationship between nine specific gut bacterial taxa and PAH [40].

Taken together, all these observations, strongly suggest that gut dysbiosis either initiates PAH or facilitates progression of already established PAH by modifying systemic immune responses (Fig. 1).

Gut dysbiosis and PAH – cause or consequence?

Chronic right heart failure (RHF) from PAH is seen in conjunction with increased intestinal congestion, reduced bowel perfusion, increased intestinal permeability and gut dysbiosis. If RHF can cause gut dysbiosis, then markers of gut dysbiosis should correlate with poor right ventricular function. The Shannon diversity index is a measure of the number of species living in a habitat (richness) and their relative abundance (evenness). There is no relationship between Shannon diversity index and various measures of right ventricular function [23]. Conversely, the Shannon diversity index correlates only with the measures of pulmonary vascular disease [23]. More importantly, modification of the gut microbiome with antibiotics or alteration of the circulating microbial metabolites in preclinical models, can prevent or regress pulmonary vascular modeling [28,29,41]. Alteration of the gut microbiome with intermittent fasting improves right ventricular function in the monocrotaline rat model of PH [42]. These clinical and preclinical data support the thinking that gut dysbiosis in PAH is less likely to be due to right ventricular failure.

Targeting gut dysbiosis to treat PAH

There is persuasive basic and clinical science evidence in favor of modulating the gut microbiome to treat PAH. Modulation of the gut microbiome may not only reduce pulmonary vascular remodeling but may also improve right ventricular function. There are several ways to regulate the gut microbiome. Fecal microbiota transplant (FMT), which involves transfer of fecal microbiota from an healthy individual into the recipient patient, has proven to be a clinically effective approach to treat patients with recurrent *Clostridioides difficile* infection [43]. FMT is also currently being evaluated as a treatment approach for patients with chronic inflammatory conditions including ulcerative colitis [44] and malignant melanoma that is resistant to immune-therapy [45,46]. Unlike treatment of recurrent *Clostridioides difficile* infection, which responds to a single treatment of fecal microbiota transplantation, to change the established dysbiotic intestinal microbiota community structure and functionality in a chronic disease, multiple sequential administrations of microbiota or preconditioning with

antibiotics will be required. This approach is called microbiota transplant therapy. Alternatively, prebiotics (oral fermentable fibers) increase serum levels of anti-inflammatory SCFAs, such as butyrate. In a recent pilot clinical trial, fermentable fiber supplementation reduced systemic blood pressure [47]. Finally, increasing intake of the anti-inflammatory bacterial metabolite butyrate (postbiotics) is an alternate option to target the gut microbiome-lung axis in PAH.

Preeclampsia and gut microbiome

Preeclampsia is a life-threatening pregnancy disorder characterized by systemic hypertension and proteinuria after 20 weeks of gestation [48]. Preeclampsia shares many factors in common with PAH. Both vascular disorders initiate with vascular endothelial cell dys-function followed by vascular stiffness and elevated vascular pressures [48]. The etiology of preeclampsia is not completely understood. Early onset preeclampsia has been linked to placental ischemia from inadequate angiogenesis leading to oxidative stress, chronic inflammation, maternal vascular endothelial dysfunction, systemic vascular stiffness, and hyper-tension [49]. Increased levels of serum lipopolysaccharide released from bacteria has been speculated to play a role in the development of preeclampsia [50]. Like PAH, interestingly, gut dysbiosis has been ass-ociated with preeclampsia. Compared to healthy individuals in early, middle, and late pregnancy, those with preeclampsia in late pregnancy have a higher relative abundance of pathogenic bacteria, *Clostridioides perfringens* and *Solobacterium moorei* and a lower relative abundance of beneficial anti-inflammatory bacteria, *Coprococcus catus* [51]. Similarly, the relative abundance of the butyrate-producing anti-inflammatory genus *Odoribacter* is inversely related to systemic blood pressure in obese pregnant patients [52]. In a two-sample Mendelian randomization study, the anti-inflammatory probiotic taxa, *Bifido-bacterium* had a protective role against development of preeclampsia [53].

Conclusion

Evolving preclinical and clinical evidence demonstrates that gut dysbiosis and altered circulating microbial metabolites are drivers of perivascular inflammation in PAH, either initiating or accelerating already established pulmonary vascular remodeling in PAH. Hence, modulating the gut microbiome is a promising novel treatment paradigm in the management of PAH.

Conflict of Interest

There is no conflict of interest.

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