Food for Thought: The Emerging Role of Intestinal Microbiota in Pulmonary Arterial Hypertension

The term "microbiome" refers to the plethora of microorganisms that live on and within an individual. Many of these microorganisms are commensal, and potentially even beneficial to the host, whereas others may contribute to disease states. The gut microbiome is of particular interest, because it can interact with molecules ingested in the diet, metabolizing them to derivatives that can be absorbed into the body. The role of the gut microbiome in cardiovascular disease (CVD) first came to the forefront about a decade ago, when metabolomic analyses of human plasma identified elevated concentrations of choline, trimethylamine *N*-oxide (TMAO) and betaine that predicted CVD risk (1). All three are metabolites of the dietary lipid phosphatidylcholine, also known as lecithin. Choline is metabolized by intestinal microbes to generate trimethylamine (TMA), a gas that is absorbed and then converted to TMAO in the liver (Figure 1). Using germ-free mice, the authors were able to demonstrate an obligate requirement for gut microbiota in the generation of TMAO from dietary phosphatidylcholine and confirmed that TMAO was only produced after oral administration, not by intraperitoneal injection. L-carnitine, another TMA-containing compound that is abundant in red meat, also promotes atherosclerosis (2). In addition, TMAO enhances platelet reactivity and risk of thrombosis (3).

In contrast to CVD, relatively little is known about whether the microbiome plays a role in pulmonary vascular disease. Shotgun metagenomic sequencing of fecal samples from patients with pulmonary arterial hypertension (PAH) versus control subjects showed significant differences in the microbiome composition, including enrichment for bacteria associated with TMA metabolism (4). The gut microbiome was also altered in the Sugen-hypoxia rat model of PAH (5). Although these studies did not directly address whether the altered microbiome is a cause or effect of pulmonary hypertension (PH), cotreatment with antibiotics significantly suppressed the elevation in right ventricular systolic pressure (RVSP) and right ventricular (RV) hypertrophy, suggesting that the microbiome may contribute to PH pathogenesis (6). In this issue of the *Journal*, Huang and colleagues (pp. 452–460) take this analysis one step further (7). First, they analyzed TMAO concentrations in plasma from 35 patients with idiopathic PAH and 19 age and sex-matched controls. Patients were stratified into low-, medium-, and high-risk groups, based on multiple clinical parameters. Plasma TMAO was significantly higher in the intermediate and high-risk patients, whereas the low-risk group did not differ from control subjects. The results in rodent models paralleled this, with a modest but nonsignificant increase in TMAO in hypoxic mice, a relatively mild model of PH, and a larger, statistically significant increase in the more severe monocrotaline rat model. 3,3-dimethyl-1-butanol (DMB) is an analog of choline that inhibits microbial TMA lyase activity, thus reducing the production of TMAO (8). Administration of DMB to hypoxic mice reduced plasma TMAO concentrations and partially suppressed the hypoxia-induced increase in RVSP but did not affect RV hypertrophy, whereas in monocrotaline rats, both RVSP and RV hypertrophy were improved (7). Conversely, addition of TMAO to drinking water increased RVSP and vascular wall thickness in the hypoxia mouse model. These results suggest that TMAO may directly contribute to the development of pulmonary hypertension and is not merely a passive biomarker of severe disease or the result of the exposures used to induce PH.

To elucidate the pathways that are modulated by DMB, Huang and colleagues performed RNA sequencing (RNAseq) of lung tissue from monocrotaline rats with or without DMB treatment. Connectivity analysis of the differentially expressed transcripts identified cytokine and chemokine signaling and complement and coagulation cascades as target pathways. However, it is not clear if these effects are mediated solely through inhibition of TMAO production or by another independent mechanism. In this regard, it would have been helpful to include a monocrotaline plus TMAO treatment group as a direct comparison, with the expectation that the same genes might be altered but in the opposite direction compared with DMB. However, some of the key genes identified from the RNAseq analysis were studied in rat bone marrow–derived macrophages treated with TMAO *in vitro*, and here the results were the opposite of DMB, as expected. Intriguingly, when studying proliferation and migration of pulmonary artery smooth muscle cells *in vitro*, TMAO had no direct effect, whereas conditioned medium from TMAO-treated bone marrow–derived macrophages stimulated increased proliferation and migration. Thus, TMAO may contribute to PH by inducing a proinflammatory environment, rather than through direct effects on pulmonary vascular cells (Figure 1).

Several questions remain to be answered, not least of which is why plasma TMAO concentrations were only elevated in moderate- to high-risk patients. Do low-risk patients naturally have a more favorable microbiome than high-risk patients, and, if so, is this stable over time? Is it related to diet? Or is there a switch to increased TMAO concentrations at some point in the disease process, which propels a low-risk patient into a higher-risk category? If so, understanding the trigger could provide new insight into managing PAH and slowing disease progression. Answering these questions will require longitudinal studies with serial analysis of plasma TMAO and fecal metagenomic sequencing at multiple time points, combined with detailed dietary information, cytokine profiling, and PAH clinical parameters. Comparing the microbiome between affected and

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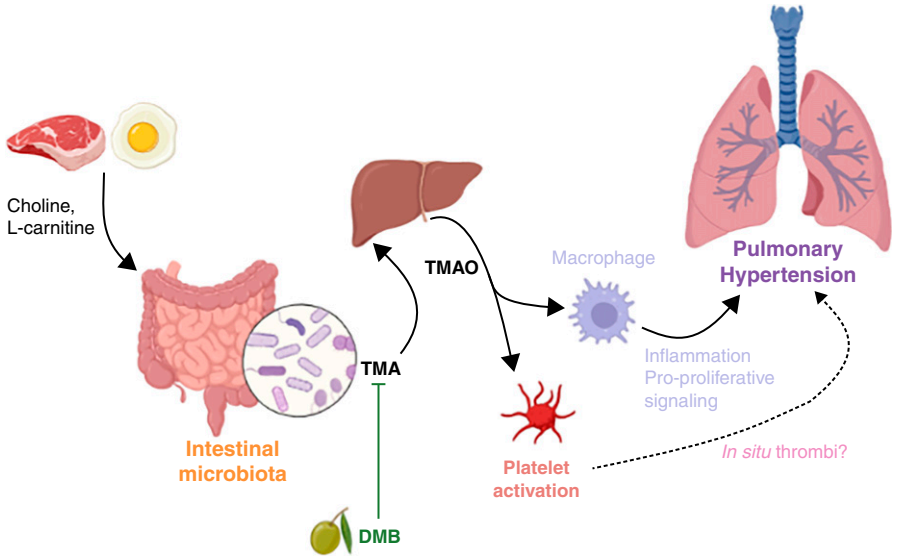

Figure 1. Graphical representation of how the intestinal microbiome might contribute to the development of pulmonary hypertension. Dietary choline and L-carnitine are catabolized to trimethylamine (TMA) by intestinal microorganisms. In the liver, TMA is metabolized to TMA *N*-oxide (TMAO). TMAO-treated macrophages have elevated cytokine expression and induce proliferation and migration of pulmonary artery smooth muscle cells (7), two of the hallmarks of pulmonary hypertension. TMAO also enhances platelet reactivity and increases the risk of thrombosis (3), which may contribute to the formation of *in situ* thrombi often seen in pulmonary hypertension lungs. However, this potential link has not been formally investigated. 3,3-dimethyl-1-butanol (DMB) inhibits microbial TMA lyase activity, thus reducing the production of TMAO, and may be beneficial in the treatment of pulmonary arterial hypertension.

unaffected mutation carriers in families with heritable PAH would also be intriguing.

Finally, is there a role for manipulating the microbiome as a PAH therapy? Foods rich in choline and L-carnitine, the precursors of TMAO, include red meat, eggs, and milk, whereas DMB occurs naturally in olive oil and red wine. The so-called Mediterranean diet, high in vegetables and olive oil but low in red meat and dairy, is already associated with lower cardiovascular risk and would probably benefit us all. Supplementing the diet with DMB might be beneficial, or even using fecal transplantation to "correct" the microbiome, but, as outlined above, we really need a clearer understanding of the timing. Would DMB be beneficial even when TMAO concentrations are normal, or only when they start to increase? More work is needed, but the data thus far definitely give us food for thought. ■

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