VIEWPOINT

The Microbiome and Risk for Atherosclerosis

Anthony L. Komaroff, MD
Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

"Hot" new areas of biomedicine sometimes generate cool skepticism. Little more than a decade ago, investigators proposed that the gut microbiome might be contributing to obesity. Since then, the microbiome has been linked to numerous major diseases, including atherosclerosis, although some have been skeptical about this association.

How could the gut microbiome influence the course of any disease? The central argument is simple and compelling. Humans actually have 2 genomes: human genes and the collective genes (the "microbiome") of the trillions of microbes (the "microbiota") that coexist with each human.

The advent of rapid nucleic acid sequencing has revealed an astonishing fact: the microbiome contains more than 100 times as many genes as there are human genes. More remarkably, these microbial genes generate proteins, including hormones, neurotransmitters, and molecules of inflammation, that can enter the circulation and affect human physiology. Thus, the microbiome is not only a second genome: it is also like an additional endocrine organ.

Considerable evidence indicates that the human gut microbiome may affect the development and progression of atherosclerosis, both by influencing risk factors for atherosclerosis and by direct effects on the initiation and progression of atherosclerotic plaques.

The Microbiome and Risk Factors for Atherosclerosis

As summarized previously, the microbiome may influence the development of both obesity and type 2 diabetes,[1] both of which are atherogenic.

Obesity

It is not the calories that people ingest that affect weight: it is the calories people digest (absorb from the gut). By increasing or decreasing the amounts of digestible sources of energy, particularly monosaccharides and short-chain fatty acids, gut bacteria affect the number of calories that humans absorb.

Consider a study of human twin pairs (mostly monozygotic), one of whom was obese. Lean mice were fed feces from the human twins. Feces from the fat twins caused lean mice to become fat, and feces from the lean twins allowed mice to remain lean. When the fat and lean mice were housed together, and ate each other's feces, the obese mice became lean and their gut flora came to resemble the flora of the lean mice (and the lean human twins).

Type 2 Diabetes

Besides the diabetogenic influence of obesity, the gut microbiome also influences an individual's risk for type 2 diabetes in other ways. For example, a microbiome that produces relatively more acetate and less butyrate increases insulin resistance, and also increases the gut's production of ghrelin (an appetite-stimulating hormone).

The gut microbiome also can promote inflammation. This, in turn, makes the gut epithelial barrier more permeable ("leaky gut") to bacterial products such as endotoxins and allows the escape of bacteria from the gut lumen into the circulation. The resulting systemic activation of the innate immune system increases insulin resistance.

One experimental study suggests that these effects on short-chain fatty acids and inflammation, demonstrated largely in rodents, also may apply to humans. Gut flora were eliminated in treatment-naive individuals with metabolic syndrome. Then, at random, the study participants received small intestinal infusions of either their own feces or feces from lean male donors. The donations from lean male donors increased the insulin sensitivity of the recipients, along with levels of butyrate-producing microbiota.

Lipid Metabolism

Cholesterol is the precursor to bile acid synthesis in the liver. The microbiome can decrease the rate of bile acid synthesis, thereby increasing levels of circulating low-density lipoprotein cholesterol.[2]

Blood Pressure

Several studies have linked gut microbiota to hypertension in rodents through effects on the angiotensin II system, and by affecting the production of short-chain fatty acids.[3] Recently, investigators reported that the microbiome may mediate the effect of a high-salt diet on hypertension. They found that particular members of the *Lactobacillus* species protected against the development of hypertension in rodents and humans, and that a high-salt diet reduced the number of these protective gut bacteria.[4]

The Microbiome and Atherosclerotic Plaques

Inflammation

Activation of the innate immune response—both within and around the atherosclerotic plaque (such as epicardial adipose tissue) and systemically—appears to enhance plaque progression and plaque rupture. When the gut microbiome triggers low-grade inflammation in the gut, allowing entry of bacteria and bacterial products into the circulation, it results in chronic systemic inflammation. In addition, some studies have found the DNA of gut bacteria within plaques, which could trigger inflammation in the plaque.

Endothelial Function

Oral and gut microbiota can affect nitric oxide signaling, and the production of hydrogen sulfide gas. Both nitric oxide and hydrogen sulfide affect vascular smooth muscle relaxation, which is of particular importance during an acute coronary syndrome.

Corresponding Author: Anthony L. Komaroff, MD, Brigham and Women's Hospital, 1620 Tremont St, Boston, MA 02120 (komaroff@hms.harvard.edu).

© 2018 American Medical Association. All rights reserved.

Downloaded From: on 05/15/2018

Trimethylamine/Trimethylamine *N*-oxide

In rodents and humans, gut microbial enzymes transform choline and L-carnitine in food into a volatile gas called trimethylamine (TMA). TMA travels through the portal circulation to the liver, where it is transformed into trimethylamine *N*-oxide (TMAO).[5]

TMAO increases platelet hyperreactivity and the formation of foam cells within plaques. It also induces multiple inflammatory proteins, such as interleukin 6, cyclooxygenase 2, E-selectin, and intercellular adhesion molecule 1. These interactions are mediated by a transcription factor that is important in orchestrating inflammation: nuclear factor kappa-light-chain-enhancer of activated B cells.[6] Antibiotic treatment that eliminates a large fraction of gut bacteria substantially reduces blood levels of TMAO.[5]

A study of more than 4000 patients undergoing coronary angiography found that, after adjustment for all major risk factors, the rate of major adverse cardiovascular events was significantly higher (adjusted hazard ratio about 1.5) among patients with the highest blood levels of TMAO compared with those with the lowest levels.[5] A systematic review including 19 prospective studies found that high levels of TMAO and TMAO precursors (L-carnitine, choline, or betaine) all were associated with a significantly increased relative risk for major adverse cardiovascular events, compared with low levels, after adjusting for cardiovascular risk factors.[7]

Might the association between the TMA/TMAO and atherosclerosis be causal? In rodents, production of TMA by gut bacteria was inhibited by a small molecule (3,3-dimethyl-1-butanol) placed in drinking water. The treatment substantially lowered TMAO levels and also prevented macrophage foam cell formation and progression of atherosclerotic plaques, without causing toxicity.[8]

Caveats

Despite the considerable evidence that the microbiome may have an important role in the initiation and progression of atherosclerosis, there also are reasons to be skeptical. First, much of the evidence is derived from animal models, and animal models of atherosclerosis do not mirror human disease in some respects. For example, in the mouse model closest to human disease—the apolipoprotein E knockout mouse with oxidized lipoproteins in the plaques—plaque rupture is not observed.

Second, not all of the published evidence is consistent with the hypothesis that increased levels of circulating TMAO are important risk factors for atherogenesis. For example, high levels of TMAO are present in fish, yet fish consumption protects against atherosclerosis. Also, a study in hamsters found that TMAO levels negatively correlated with a molecular surrogate for atheroma formation. There is a carnitine paradox, too: L-carnitine supplementation in male mice resulted in fewer and smaller aortic root atheromas.

While the bacterial microbiome has been studied extensively in atherosclerosis, very little is known about the virome. This could be important given recent discoveries that gut viruses—particularly bacteriophage—are ubiquitous and, like bacteria, capable of producing hormones that affect mammalian physiology.

Therapeutic Implications

Studies suggesting that the microbiome may be another modifiable risk factor for atherosclerosis raise the hope for novel effective therapies, such as probiotics or prebiotics, antibiotics, or small molecules that target the bacterial enzymes. However, the understanding of the microbiome is in its infancy, and the day of effective microbiome-based treatment is in the distant future. At this point, the most important conclusion to draw from microbiome research is that the phenotype of disease is the result of a constant interaction between human genes and environmental factors (including microbial genes).[9]

Conclusions

Discoverers often fail to grasp the magnitude of their discovery. When van Leeuwenhoek built his microscope and discovered that lake water was "alive with…multifarious animalcules," he did not imagine that such creatures might have a role in causing disease.[10] When, more than a century later, Pasteur, Koch, and others discovered a few microbes that did cause disease, they did not imagine that "commensal" microbiota in humans were anything more than freeloaders, living off nutrients in the body. In contrast, it now appears increasingly likely that these commensal microbiota have a large influence on human health. How large? Perhaps, it is hard to imagine.

ARTICLE INFORMATION

Published Online: May 14, 2018. doi:10.1001/jama.2018.5240

Conflict of Interest Disclosures: The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Additional Contributions: Joseph Loscalzo, MD, PhD (Brigham and Women's Hospital, Harvard Medical School), provided valuable comments and received no compensation.

REFERENCES

1. Komaroff AL. The microbiome and risk for obesity and diabetes. *JAMA*. 2017;317(4):355-356.

2. Sayin SI, Wahlström A, Felin J, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab*. 2013;17(2):225-235.

3. Pluznick JL, Protzko RJ, Gevorgyan H, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U S A*. 2013;110(11):4410-4415.

4. Wilck N, Matus MG, Kearney SM, et al. Salt-responsive gut commensal modulates T_H17 axis and disease. *Nature*. 2017;551(7682):585-589.

5. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368(17):1575-1584.

6. Li DY, Tang WHW. Gut microbiota and atherosclerosis. *Curr Atheroscler Rep*. 2017;19(10):39.

7. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of prospective studies. *J Am Heart Assoc*. 2017;6(7):e004947.

8. Wang Z, Roberts AB, Buffa JA, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell*. 2015;163(7):1585-1595.

9. Loscalzo J. Gut microbiota, the genome, and diet in atherogenesis. *N Engl J Med*. 2013;368(17):1647-1649.

10. Boorstin DJ. *The Discoverers*. New York, NY: Random House; 1983:330-331.

© 2018 American Medical Association. All rights reserved.

Downloaded From: on 05/15/2018