Debugging Obesity-related Airway Hyperresponsiveness by Modulating the Microbiome

The global obesity epidemic is responsible for diverse health complications that confront providers in every medical specialty. The respiratory system is especially impacted by obesity, which causes obese individuals to experience shortness of breath and other symptoms that lead to some receiving a diagnosis of asthma (1, 2). For obese patients with the most severe form of asthma (sometimes termed "inherent asthma" or "innate asthma"), there are limited treatment options outside of lifestyle alterations or bariatric surgery to induce weight loss (3). Preclinical mouse models of genetic- or diet-induced obese asthma have provided mechanistic insight into the innate immune pathways that contribute to this disease and its exacerbation, and have also provided compelling evidence for the importance of the gut bacterial microbiome. Specifically, there is an obesogenic microbiome in people and mice, there is an alteration in the microbiome during weight loss, and dietary factors can alter the normal microbiome. Consequently, manipulating the microbiome in obesity through methods such as therapeutic replacement and dietary supplementation (e.g., with prebiotics that enable beneficial microbes to thrive) offers an alluring objective for translational research. Few papers, however, have reported on the effects of the gut microbiome and its manipulation on baseline and exacerbation-induced methacholine responsiveness in mouse models of obese asthma.

In this issue of the *Journal,* the paper by Tashiro and colleagues (pp. 702–712) describes three studies that investigated the contribution of the gut microbiome to ozone-induced exacerbation of innate airway hyperresponsiveness in a genetic model of obesity (4). The authors present qualitative and quantitative information on the gut microbiome alterations seen in the leptin receptor-mutant *db/db* model of obesity (showing hyperphagia, reduced energy expenditure, and hyperglycemia) (5), upon transfer of colonic (fecal) contents from wild-type and *db/db* mice to germ-free recipient mice, and in *db/db* mice provided diets with augmented fermentable or nonfermentable fiber (pectin or cellulose, respectively). Also presented are assessments of inhaled methacholine responsiveness and pulmonary inflammatory markers from air- and ozone-exposed lean and obese mice from the aforementioned groups.

In the first study, broad-spectrum antibiotic cocktail treatment for 2 weeks did not influence body weight, but it did decrease the ozone-induced methacholine hyperresponsiveness of *db/db* mice while having no effect on inflammatory outcome measures. In the second study, germ-free mice reconstituted with the gut microbiome from *db/db* donors displayed augmented ozone-induced methacholine hyperresponsiveness and lung inflammatory outcomes compared with mice that received colonic contents from wild-type donors. In the third study, *db/db* mice provided with supplemental pectin for 3 days displayed reduced ozone-induced methacholine hyperresponsiveness and lung inflammation compared with mice provided supplemental cellulose. Especially decreased in the BAL fluid of pectin-supplemented *db/db* mice were BAL neutrophils and IL-17A, the latter of which is a cytokine linked to obesity-associated methacholine hyperresponsiveness (6, 7).

On the microbiology front, the first study revealed that the gut microbiota (as assessed by 16S rRNA sequencing of fecal DNA) was modestly distinct, as determined by a principal coordinate analysis, between wild-type and *db/db* mice before antibiotic administration, with a predominance of Firmicutes at the phylum level. In the second study, the gut bacteria recovered after reconstitution of germ-free mice with the colonic contents from either wild-type or *db/db* mice, and showed a predominance of Bacteroidetes along with an expanded Verrucomicrobia and contracted Firmicutes composition. Nevertheless, a principal coordinate analysis again revealed differences between the two groups, which are attributable to differences in the lower-abundance taxa. In the third study, there were substantial differences in the gut bacteria as a consequence of feeding cellulose- or pectin-enriched diets. The *db/db* mice displayed a markedly expanded abundance of Proteobacteria and *Enterobacteriacae*, in addition to reduced Verrucomicrobia, upon pectin supplementation.

It is important to note that the gut microbial communities established in the germ-free host mice after transfer of colonic contents from wild-type or *db/db* mice were not the same as those that were present in the donor mice. This suggests that altering the microbiota (causing dysbiosis) itself, not necessarily making its composition more like that of wild-type mice, was in some instances sufficient to induce protective effects against ozone-induced methacholine hyperresponsiveness. Not performed in the current studies were experiments to determine whether establishing a wild-type gut microbial community in *db/db* mice would decrease ozone-induced methacholine hyperresponsiveness. Interestingly, the studies that demonstrated a benefit to methacholine hyperresponsiveness did so without reducing body weight. Bariatric surgery and associated weight loss are of clinical benefit to obese individuals with asthma (8). Whereas there is certainly an influence of body mass *per se* on obese asthma, it seems that the benefits to methacholine hyperresponsiveness elicited in these mouse studies before weight loss point to additional mediators of pathology that are affected by diet and the microbiome. It remains unclear what portion of the observed effects are a consequence of transient dysbiosis and its accompanying acute impacts on the host (e.g., gut transit time, provision of bacteria-derived nutrients, immune modulation, and nervous system stimulation) versus the establishment of a

stable, "healthy" gut microbiome that would provide a durable benefit.

A goal of animal modeling is to provide evidence to translate promising findings into human studies. IL-17A has been implicated as a causal factor in ozone-induced methacholine hyperresponsiveness in lean (9) and obese (6, 7) mice, and biologics targeting this cytokine are being used for the treatment of psoriasis, psoriatic arthritis, and ankylosing spondylitis. Fecal microbiota transplantation is used for recurrent *Clostridioides difficile* infection, and it was hoped that it could be applied to other chronic diseases, including allergic and obese asthma. However, biologics and fecal microbiota transplantation are costly, whereas the consumption of prebiotics could easily and much more economically be implemented, provided that the levels used in mice can be adapted to people. The amount of cellulose in the cellulose-enriched diet was considerably higher (30% by weight) than that in conventional mouse chow (~4%), and the amount of pectin in the pectin-enriched diet (30% by weight) is in stark contrast to its absence from the conventional chow. The most recent U.S. recommendations for human consumption of dietary fiber are 25 g/d and 38 g/d for women and men, respectively (10). As people typically consume three to four pounds (1,361–1,814 g) of food daily, the recommended amount of dietary fiber consumption constitutes ~1.7–2.9% of intake, which is considerably lower than that consumed by the mice on the fiber-supplemented diets. Whether humans could consume an amount of fermentable fiber sufficient to elicit the beneficial effects on methacholine hyperresponsiveness seen in the mice remains to be determined. Another question that remains is, how durable are the effects of any of the approaches studied in this paper? Would the benefit recede as the pathogenic microbiota is reestablished? Fermentable fiber has been considered as a source of bacterial metabolites, including short-chain fatty acids (SCFAs), that can ameliorate allergy-associated hyperresponsiveness (11). In follow-up studies, it will be interesting to see whether the circulating levels of acetate, propionate, and butyrate are augmented in pectin-supplemented *db/db* mice, and whether they are sufficient to decrease ozone-induced methacholine responsiveness in obese mice. Importantly, it seems that the effects of SCFAs may be context dependent, as pectin or SCFA supplementation in normal-weight mice was reported to increase ozone-induced methacholine hyperresponsiveness (12).

Overall, the clearly presented data and appropriately interpreted results indicate that gut microbes contribute to obesity-associated methacholine hyperresponsiveness and are responsive to dietary modulation with fermentable fiber, which may provide a potential treatment for obesity-associated asthma. This impactful work complements the field and may provide guidance for future human studies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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