ADVANCES IN HEPATOLOGY

Current Developments in the Treatment of Hepatitis and Hepatobiliary Disease

Section Editor: Eugene R. Schiff, MD

Update on the Role of the Gut Microbiota on Alcohol-Associated Liver Disease

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G&H What is the current understanding of the relationship between the gut microbiota and alcohol-associated liver disease?

BS Alcohol-associated liver disease is accompanied by changes in the gut microbiota. Compositional change in the bacterial microbiota includes a proportional decrease in beneficial bacteria and an increase of possible pathogenic bacteria, also called pathobionts, in the gut. The gut microbiota not only consists of bacteria, but also contains fungi (fungal mycobiome) and viruses (virome). Recent work showed that patients with alcohol use disorder (with or without liver disease) and patients with alcohol-associated hepatitis have a decrease in fungal diversity and a proportional increase in *Candida* species, including *Candida albicans*. It is interesting that these changes appear to be related to chronic alcohol consumption rather than the liver disease stage itself. Two weeks of alcohol abstinence reversed many of the fungal mycobiome changes and resulted in a lower abundance of *C albicans* in patients with alcohol use disorder. The specific anti–*C albicans* immunoglobulin G (IgG) levels were increased in patients with alcohol use disorder as compared with nonalcoholic controls. Abstinence for 2 weeks resulted in a significant reduction of anti–*C albicans* IgG levels, indicating that an increased immune response to fungi is also reversible with alcohol abstinence. Furthermore, the fecal virome was recently characterized in patients with alcohol-associated hepatitis. The gut virome consists mainly of bacteriophages (also called phages), which are small viruses that can infect, replicate inside, and lyse (kill) specific bacteria. The phage profile is different in patients with alcohol use disorder (with or without liver disease) and patients with alcohol-associated hepatitis as compared with nonalcoholic controls. Most strikingly, patients with alcohol-associated hepatitis had a significant increase in mammalian viruses in the stool, which included viruses from the families *Herpesviridae* (in particular Epstein-Barr virus) and *Parvoviridae*. It is currently not clear why these viruses can be detected in the stool and whether they contribute to liver disease progression.

G&H Have there been any novel discoveries linking the microbiome to alcohol-associated liver disease?

BS My colleagues and I recently discovered a toxin, cytolysin, that is secreted specifically by *Enterococcus faecalis*. We found that approximately 30% of patients with alcohol-associated hepatitis tested positive for cytolysin-producing *E faecalis* in the stool, whereas nonalcoholic controls tested negative for cytolysin and only 1 patient with alcohol use disorder was cytolysin-positive. We further demonstrated that cytolysin positivity strongly correlates with mortality; 89% of cytolysin-positive patients with alcohol-associated hepatitis died within 180 days,

whereas only 3.8% of cytolysin-negative patients with alcohol-associated hepatitis died within 180 days following hospital admission. Colonization of patients with cytolysin-positive *E faecalis* appears to be specific for alcohol-associated hepatitis, as a cohort of patients with nonalcoholic fatty liver disease showed a low positivity rate for cytolysin-positive *E faecalis*, and no correlation was found with liver disease severity. This indicates that cytolysin is a good biomarker for alcohol-associated hepatitis, but not a general biomarker for liver disease severity. Furthermore, we were able to demonstrate that cytolysin-positive *E faecalis* exacerbate ethanol-induced liver disease in mice. Cytolysin acts as a direct toxin for liver cells, likely through a mechanism involving pore formation in the cell membrane.

G&H Based on the research conducted thus far, can probiotics or prebiotics be used to alter, reverse, or otherwise manage alcohol-associated liver disease?

BS In general, probiotics have shown improvement in direct and indirect markers of disease severity in patients with alcohol-associated liver disease. However, most studies have a small sample size, have a heterogeneous trial design, and are rarely reproduced. Thus, no clear recommendation can be made for the use of prebiotics or probiotics in patients with alcohol-associated liver disease. Probiotics appear to be safe even when administered to patients with cirrhosis.

Preclinical models have evaluated the benefit of engineered bacteria. These next-generation probiotic bacteria were engineered to have increased beneficial effects as compared with their parent isogenic probiotic strain. An example is *Lactobacillus reuteri* producing interleukin-22, which is a gut barrier protective cytokine. Engineered interleukin-22 secreting *L reuteri* increased antimicrobial molecule expression, reduced bacterial translocation to the liver, and improved ethanol-induced liver disease in a preclinical model. This is an elegant example of how restoring the gut barrier protects mice from liver disease. Probiotic bacteria can also replenish bacteria that have been depleted during disease. Another beneficial effect of probiotics is that they provide resistance against colonization of pathogenic bacteria.

G&H Thus far, does there appear to be a role for fecal microbiota transplantation in the management of liver disease?

BS Fecal microbiota transplantation (FMT) is replacing an individual's microbiota with the entire microbial community of a healthy donor. FMT has great success in curing diarrhea caused by *Clostridioides difficile*. One small pilot study using daily FMT administered through a nasoduodenal tube in corticosteroid-ineligible patients with severe alcoholic hepatitis (n=8) for 7 days showed a survival benefit as compared with a historical control group (n=8). This study was conducted in India, and patients with severe alcohol-associated hepatitis had a very high mortality rate. It remains to be seen whether the results can be confirmed in patients with severe alcohol-associated hepatitis from Europe or North America, where the mortality rates are lower.

In addition, 2 small phase 1 clinical safety trials were conducted in patients with cirrhosis and recurrent hepatic encephalopathy. One trial used a single FMT enema following antibiotic treatment (n=10) vs standard of care (n=10), and the second one used 15 FMT capsules (n=10) vs placebo (n=10). FMT in both trials was well tolerated and improved intestinal dysbiosis and hepatic encephalopathy.

In a phase 1, double-blind, randomized clinical trial, patients with alcohol-related cirrhosis with problem drinking received 1 placebo (n=10) or FMT enema (n=10) from a rationally chosen donor. The donor was selected based on the microbial composition of the stool. FMT led to short-term reduction in alcohol craving and consumption, improved microbial dysbiosis, and was well tolerated.

G&H What are the potential risks and limitations of this approach?

BS Although the aforementioned small clinical trials appeared to be safe, a recently published case report described 2 patients in whom extended-spectrum beta-lactamase–producing *Escherichia coli* bacteremia occurred after they had undergone FMT. One of these patients had cirrhosis and received oral capsules to treat refractory hepatic encephalopathy in an open-label trial of FMT. Thus, careful screening needs to be implemented of the healthy individuals who are serving as stool donors. In addition, many liver diseases are chronic, so future studies need to evaluate whether FMT needs to be administered repeatedly. The use of oral FMT capsules will allow for an easier administration of stool as compared with endoscopically administered FMT.

G&H Have any other agents or approaches been studied to modify the gut microbiota to treat liver disease?

BS Phages can specifically recognize bacterial strains, replicate inside, and finally destroy them. Newly assembled phages can target and kill other bacteria, which is an efficient and self-perpetuating system of prey and

predator. As previously mentioned, my colleagues and I used phages to target cytolysin-positive *E faecalis* in a humanized preclinical model. Lowering cytolysin-positive *E faecalis* in the intestine reduced ethanol-induced liver disease in mice. Phages are ubiquitous microbes that are present in a large quantity in our intestines. They are generally considered safe even when administered intravenously to target multidrug-resistant bacteria. Obstacles for widespread use of phages have to do with pharmacokinetics and pharmacodynamics. In addition, phages have a narrow host range, and bacteria can develop resistance to phages, which can be overcome by using a cocktail of multiple phages.

G&H What is the role of precision medicine in this area?

BS Microbiome-targeted therapies need to become personalized. Not every patient with alcohol-associated liver disease might have the same changes in the gut microbiota; for example, liver disease in some patients might be more driven by fungal dysbiosis and other patients by bacterial dysbiosis. As mentioned earlier, only 30% of patients with alcohol-associated hepatitis are cytolysin-positive, and these are the patients at highest risk of dying. Success of any microbiome-centered therapy requires screening of patients for the presence and abundance of the target. With respect to phage therapy, cytolysin-positive *E faecalis* need to be present in the stool and susceptible to phages. A personalized treatment approach with precision therapy will result in a powerful combination to treat not only alcohol-associated liver disease, but many other chronic liver diseases.

G&H What are the next steps in research?

BS Research about the microbiome is still in its infancy. Although we can now identify the majority of bacteria, the function of many of them is still elusive. More studies about fungi and phages and their involvement in liver disease are required. We also do not understand the interactions between the microbes very well. Furthermore, we need to research microbial metabolites and how they contribute to liver disease. All of these aspects will hopefully allow for a better understanding of the dynamic interactions between the microbes and the host.

Acknowledgements

Dr Schnabl is supported by NIH center P30 DK120515.

Disclosures

Dr Schnabl has been consulting for Ferring Research Institute, HOST Therabiomics, Intercept Pharmaceuticals, Mabwell Therapeutics, Patara Pharmaceuticals, and Takeda. Dr Schnabl's institution UC San Diego has received research support from Axial Biotherapeutics, BiomX, CymaBay Therapeutics, NGM Biopharmaceuticals, Prodigy Biotech, and Synlogic Operating Company. Dr Schnabl is founder of Nterica Bio. UC San Diego has filed several patents with Dr Schnabl as inventor related to this work.

Suggested Reading

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