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Intestinal microbiota and its host: harmony or discord?

Intestinal microbiota in liver diseases

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INTRODUCTION

Overweight, obesity and alcohol consumption are the leading causes of liver disease in Western countries. Hepatic lesions induced by these nutritional factors are found at different frequencies and at different levels of severity in clinical practice. The intestinal microbiota is now widely considered as a potential factor in individual susceptibility to liver diseases, especially in those related to alcohol consumption. Several recent studies have been carried out to identify its action, its impact, and the mechanisms by which its activity on metabolism is exerted.[1-5] The mechanisms related to the modulation of the gut microbiota are now better understood, having been shown by two studies that also highlighted the major role of probiotics and prebiotics in this modulation.[6,7] These data demonstrate the strong relationship between intestinal microbiota and liver disease, and suggest that the gut microbiota may be both a new diagnostic marker and a therapeutic target for liver disease.[8-10]

HEPATIC LESIONS IN NUTRITIONAL DISEASES OF THE LIVER

In patients with diet-, overweight- or alcohol-related liver disease, the range of hepatic lesions includes "simple" steatosis (non-alcoholic steatohepatitis [NASH]), hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma. In 20% of patients, however, the disease progresses to more severe stages.

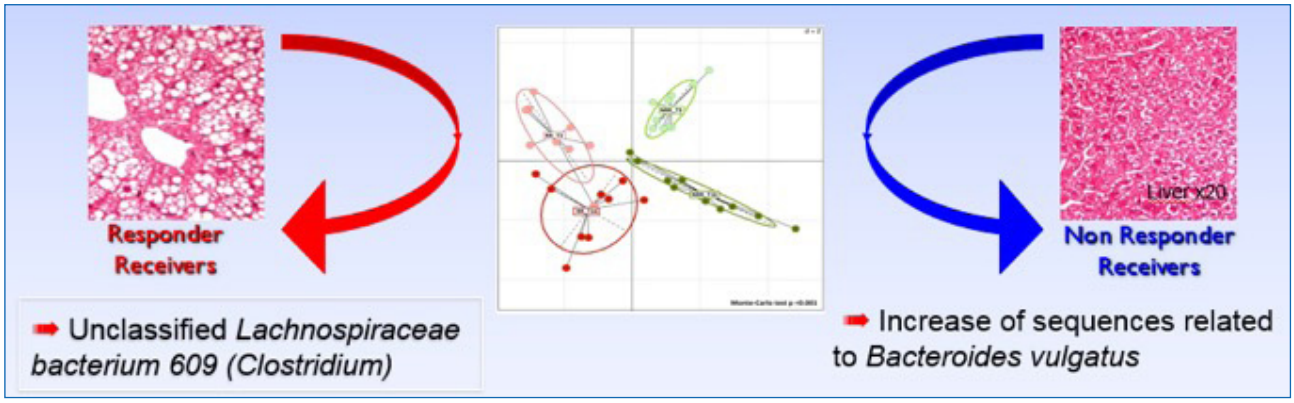
MICROBIOTA AND STEATOSIS: THE VARIOUS IMPLICATIONS OF INTESTINAL MICROBIOTA IN HEPATIC DISEASE

Surprisingly, among people with excessive alcohol consumption some are better off than others. In fact, while some patients suffer from severe liver disease related to their over-consumption of alcohol, others with higher consumption are healthier. It appears, therefore, that regardless of the amount of alcohol consumed, some excessive users will suffer from alcoholic liver disease (ALD) while others will not, suggesting the presence of important cofactors in ALD genesis; one of them is the gut microbiota.[11]

Steatosis is an accumulation of triglycerides in the cytoplasm of hepatocytes. In an experiment carried out on mouse models provided by the same animal center and with the same genetic heritage, it was observed that the same diet did not result in weight gain in some mice while others experienced considerable weight increase. Among these, several mice showed no insulin resistance, while others had both insulin resistance and inflammatory syndrome.[12]

In order to specify the role of the microbiota in this difference, the stools of these mice were implanted in axenic mice which were subsequently given a high-fat diet. It was observed that the phenotype was transferred to the axenic mice: those who received the intestinal microbiota of mice that had developed diabetes and insulin resistance also exhibited insulin resistance, and steatosis. This finding shows the causal role of the gut microbiota in metabolic steatopathy (Figure 1).[12]

Figure 1. Dysregulation of liver genes involved in lipid metabolism[12]



MICROBIOTA AND INFLAMMATION IN ALCOHOLIC LIVER DISEASE

Severe acute alcoholic hepatitis is a particular phenotype of ALD found in 5% of patients who consume alcohol. This type of severe hepatitis, which induces short-term vital risk (even when alcohol consumption is stopped), is an inflammatory disease that must be treated with cortisone to improve survival.

Ethanol production by the microbiota

In the overweight subject, ethanol is produced by the gut microbiota. This ethanol then passes from the digestive tract to the portal vein and activates the Kupffer cells. It provokes a proinflammatory phenotype in the liver and contributes to the genesis of obesity-related NASH. A study by Cope et al[13] showed that in this context, antibiotics decrease the production of ethanol in the digestive tract in case of carbohydrate-enriched diet. A study was recently conducted in obese patients to identify the relationship (via the gut microbiota) between endogenous alcohol and NASH.[14] The authors state in conclusion that the overproduction of ethanol-producing bacteria in the microbiota, the higher concentration of ethanol in the blood of NASH patients, and the well-established role of alcohol metabolism in oxidative stress (inducing inflammation of the liver), evoke alcohol production by the microbiota in the pathogenesis of NASH.[14]

A study by Ciocan et al[15] carried out in severe alcoholic hepatitis reveals a specific dysbiosis. The authors consider this dysbiosis related to an increase of actinobacteria, gamma-proteobacteria and bacilli in patients with severe alcoholic hepatitis, and conversely, they report an increase of bacteroidetes and alpha- and delta-proteobacteria in patients without alcoholic hepatitis.

Microbiota transfer in murine models

To determine whether in patients with severe alcoholic hepatitis the dysbiosis is responsible for susceptibility to ALD or if it is a consequence of ALD, microbiota from patients consuming alcohol and developing or not developing ALD were transferred to germ-free mice.[16] The authors report: 1) that susceptibility to the development of ALD was transmissible via the gut microbiota, and 2) that the microbiota of a person consuming alcohol but free of liver disease did not induce ALD in the recipient mouse and improved the liver phenotype.[16] Thus, individual sensitivity to alcohol is closely related to the gut microbiota, of which the causal role in ALD has been verified.

The corresponding mechanism can be identified by metabolomic analysis. Llopis et al[16] continued their experiment by analyzing the fecal metabolic profile of two groups of mice (with and without ALD) and observed a specific metabolomic profile in both groups. One of the most discriminating metabolites was UDCA (ursodeoxycholic acid), an anti-inflammatory, postbiotic bile acid, of which the concentration was increased in mice without ALD.

A more in-depth examination of bile acid metabolism was performed on mice with deficiency of TGR5, one of the bile acid receptors.[17] The TGR5-deficient mouse developed more severe ALD with ethanol and showed a specific dysbiosis. When this dysbiosis of TGR5-deficient mice was transferred to non-TGR5-deficient mice, administration of ethanol to recipient mice resulted in more severe disease when they had dysbiosis, showing involvement of bile acid metabolism and the major role of bile acids in immunity, as in liver diseases and inflammation.

Alcohol metabolism in mice

In order to determine the metabolic behavior of mice fed with alcohol, a model of alcohol consumption was developed, with mice from two different centers receiving increasing doses of alcohol (up to 5%).[18] The authors of this experiment report that the mice of animal facility A did not develop hepatic lesions, unlike the mice of animal facility B, which developed high steatosis and inflammation; hepatic injury was associated with a decrease in bacteroides.[18]

Fecal transfer and pectin treatment

In order to determine if maintaining bacteroides at a high level could prevent liver damage, the following treatments were added to the alcohol diet of B mice, so as to maintain high levels of bacteroides: 1) fecal therapy consisting of transplanting the microbiota of mice A without liver lesions and with high bacteroides levels, 2) addition of pectin to the diet, a prebiotic that promotes the growth of bacteroides.

These two treatments actually increased bacteroides levels. It was then verified that maintaining high levels of bacteroides was likely to prevent steatosis and liver damage. A decrease in the amount of triglycerides and ALATs (alanine aminotransferases) was observed. This experiment demonstrates that maintaining high levels of bacteroides can reverse the alcohol-related proinflammatory phenotype and prevent the onset of liver damage.[18]

THE MECHANISMS

Microbiota transfer and pectin administration induce a change in mucus phenotype. They act on the intestinal epithelium by increasing the number of goblet cells in the digestive tract and a high amount of antibacterial peptides, thus improving the mucus layer.[18]

Impact of *Akkermansia* on the mucus layer

A study by Grander et al[19] showed that in both mice and humans, exposure to ethanol reduces the amount of *Akkermansia muciniphila* in the gut. This amount can, however, be restored by oral supplements in experimental ALD. In fact, *Akkermansia muciniphila* preserves the integrity of the intestinal barrier via an increase of the protective mucus layer. The result is the improvement of liver disease, suggesting that patients with ALD may benefit from *Akkermansia muciniphila* supplements.

MICROBIOTA AND HEPATIC FIBROSIS

Blood microbiota and fibrosis in NASH

In a study aimed at observing the relationship between blood microbiota and hepatic fibrosis in European cohorts of patients with severe obesity, the potential role of bacterial translocation in liver disease and obesity was verified. 16S sequencing revealed specific differences regarding the amount of several blood and fecal bacterial taxa correlated with the presence of liver fibrosis, defining a specific signature of liver disease. Changes in the blood microbiota, therefore, appear to be associated with hepatic fibrosis in obese patients.[20]

Blood microbiota and fibrosis in ALD

Phenotyping the circulating microbiota makes it possible to identify patients with fibrosing liver disease related to their being overweight as compared with those without fibrosis. This has also been suggested in patients with ALD: there is a different phenotype of the circulating microbiome depending on the severity of ALD.[21-23]

INTESTINAL MICROBIOTA, CIRRHOSIS AND HEPATITIS

Clinical perspectives: dysbiosis/cirrhosis ratio

The intestinal microbiome is altered in cirrhosis. However, its evolution during the course of the disease is only partially understood. A study has analyzed the changes that occur in the microbiome in relation to the severity of cirrhosis, its stability over time and its longitudinal alterations with decompensation.[24] The study results show progressive changes in the intestinal microbiome, which accompany cirrhosis and worsen in case of decompensation. The dysbiosis/cirrhosis ratio may be a quantitative index that could be useful in the assessment of microbiome alterations associated with the progression of cirrhosis.[24]

Primary biliary cholangitis

Primary biliary cholangitis (PBC) is a chronic, idiopathic and fibro-inflammatory cholangiopathy. The role of the microbiota in the pathophysiology of PBC is poorly understood. A study carried out using germ-free mouse models shows that the absence of bacteria induced exacerbation of biliary lesions and increased senescence of cholangiocytes, a potential hallmark of progressive biliary disease.[25] UDCA, which is a secondary bile acid that is lacking in germ-free mice, improves cholangiocytes. These results emphasize the importance of the microbiota and its metabolites in protection against biliary lesions. They suggest potential subjects for further research on biomarkers and for therapeutic interventions in primary cholangitis.[25]

Fecal transplantation and hepatitis B

A pilot study showed that microbiota transfer in patients with chronic hepatitis B not responding adequately to antiviral treatments, could alter immunity and improve the response to antiviral therapy.[26]

INTESTINAL MICROBIOTA AND HEPATOCELLULAR CARCINOMA

Indirect evidence

The increased translocation of intestinal bacteria is a hallmark of chronic hepatitis and contributes to inflammation and liver fibrosis. Dapito et al[27] have tested in mouse models the hypothesis that the intestinal microbiota and Toll-like receptors (TLRs) have a role in hepatic carcinogenesis. The results of this study show that the lipopolysaccharide (LPS) and its receptor TLR4 promote hepatocellular carcinoma. They also show that TLR4 deficiency protects against this disease, as antibiotics do in the later stages of carcinogenesis. This implies that intestinal microbiota and TLR4 may constitute therapeutic targets for hepatocellular carcinoma in liver diseases.[27]

Expression of TLRs 3, 4 and 9 and survival in hepatocellular carcinoma

TLRs are of major interest in cancer research owing to their role in several biological processes: innate immune responses, induction of adaptive immune responses, regulation of inflammation, recovery, and carcinogenesis. A study conducted to evaluate the expression and clinical relevance of TLR3, 4 and 9 in hepatocellular carcinoma shows an association between TLR3, TLR4 and TLR9 expression, tumor aggressiveness, and poor prognosis in this disease.[28]

Biliary acids, microbiome and hepatocellular carcinoma

The gut microbiota appears to be involved in the development of liver cancer via the metabolism of bile acids that modulate hepatic immunity.

CONCLUSION

Various studies confirm, in experimental models as well as in humans, the existence of a strong relationship between the gut microbiota and liver diseases, especially those related to alcohol consumption. The mechanisms of microbiota activity on metabolism, such as those involved in its modulation, are now better understood and suggest that the intestinal microbiota could be a therapeutic target in liver diseases.

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