Liver tissue microbiome in NAFLD: next step in understanding the gut–liver axis?

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The pathogenesis of non-alcoholic fatty liver disease (NAFLD), the most common global liver disease, is complex as multiple parallel hits drive this disorder. Whereas lipotoxicity, insulin resistance, inflammatory processes, oxidative stress and others reflect key components, studies from the past years have clearly shown that the gut microbiome might also substantially contribute to the evolution of NAFLD including its inflammatory component, that is, non-alcoholic steatohepatitis (NASH). Several studies from the last years have revealed alterations in the gut microbiome in people with NAFLD compared with healthy controls. NAFLD subjects accompanied by advanced fibrosis exhibit a gut microbiome signature with increased concentrations of Proteobacteria and *Escherichia coli*.[1] Gut bacteria-derived metabolites such as phenylacetic acid and endotoxin are associated with degree of steatosis in female NAFLD patients and this metabolite, and the microbiota from NAFLD patients, induced hepatic lipid accumulation when given to mice.[2] Therefore, evidence is increasing that the intestinal microbiota and its metabolites might play a crucial role in the pathogenesis of liver diseases including NAFLD.[3]

The liver is the key gatekeeper of blood flow from the portal vein draining the intestine and is constantly challenged by intestine-derived bacteria and bacterial components, such as endotoxin and other soluble molecules. Importantly, an intact intestinal epithelial barrier might protect the liver from bacterial challenges. In the liver, Kupffer cells, prototypic tissue-resident macrophages, are highly phagocytic and efficiently clear blood-borne pathogens including bacteria arriving from the gut via the portal vein or through the oral cavity. Their location within hepatic sinusoids perfectly enables them to eliminate pathogens and other components entering the liver including cellular debris. The liver capsule seems also to play a role in host defense as capsular monocyte-derived macrophages limit hepatic dissemination of intraperitoneal bacteria.[4] It is not well established under which circumstances liver defense mechanisms start failing to handle bacterial components especially in case of an impaired gut epithelial barrier. Considering the well-known gut barrier dysfunction and intestinal immune defects in NAFLD and other liver disorders it is conceivable that bacteria or bacterial antigens enter the portal vein, the liver and the circulation.

Indeed, microbial DNA has been detected in some studies in the liver and in the circulation. For example, the presence of tissue-specific bacterial DNA has been demonstrated in mice in various compartments including liver, adipose tissue, heart, brain and muscle.[5] Furthermore, *microbial 16S rRNA genes* assessed in blood of participants from the Epidemiology Study on the Insulin Resistance Syndrome correlated with risk of diabetes[6] and with cardiovascular complications and the detection of Proteobacteria, rather proinflammatory strains.[7] A 16S *rRNA gene* signature was also demonstrated in leucocyte and platelet fractions from whole blood of healthy humans but not in plasma.[8] A blood 16S *rRNA gene* signature was also found in a small NAFLD cohort investigating obese subjects with fibrosis.[9] In *Gut*, Sookoian and colleagues went a step further by studying the liver tissue 16S *rRNA gene* bacterial metataxonomic signature in two cohorts of NAFLD patients.[10] Patients with severe obesity differed in their liver bacterial DNA profile compared with a group with overweight/moderate obesity. As demonstrated in other NAFLD studies when assessing the gut microbiome,[1] *the proportion of liver 16S rRNA genes* from Proteobacteria, particularly in the Gamma class, was increased in severe obesity, whereas in case of moderate obesity Gamma and Alphaproteobacteria as well as *Deinococcus-Thermus* dominated. A decrease in liver bacterial DNA from the Lachnospiraceae family was associated with histological severity, that is, presence of NASH. Interestingly, several members of the Lachnospiraceae produce butyrate, which is essential for homoeostasis at the colonic epithelium and prevents the expansion of facultative anaerobic bacteria, such as Proteobacteria.[11] Authors also demonstrated an increase in endotoxin in the portal tract in NAFLD subjects, a finding which was recently also reported from another study.[12] Unfortunately, in this study *faecal and oral microbiomes have* not been assessed, and therefore the questions remain about (1) the origin of the bacterial DNA present in the liver, since the oral cavity is a major site of bacterial translocation and continuous infusion of bacteria from the parodontal tissue occurs in 50% of patients over 50 years old; (2) the possibility that the liver *bacterial DNA* signature could reflect mechanisms of intestinal gut microbiota dysbiosis and epithelial dysfunction. Notwithstanding, Sookoian and colleagues concluded that liver tissue in NAFLD subjects contains substantial amounts of bacterial DNA correlating with histological disease severity.

The field of tissue metataxonomics is attracting increasing interest and several studies reported detection of bacterial components in liver tissues. Certain commensals such as *Fusobacterium nucleatum* play potentially a role in colorectal carcinoma and this bacterium was detected in liver metastases in a high number of subjects.[13] Another exciting study observed the presence of a specific pathobiont (*Enterococcus gallinarum*) in liver tissue thereby triggering autoimmunity similar to systemic lupus erythematosus (SLE).[14] Treatment of lupus mice with vancomycin enhanced life span and decreased antibodies against SLE autoantigens. *E. gallinarum* could also be detected in livers of patients with autoimmune hepatitis.[14] Certain commensals could change their behaviour and become detrimental to its host as recently shown for *Enterococcus faecalis* in alcoholic hepatitis.[15] In this study, intestinal presence of *E. faecalis* correlated with overall mortality in alcoholic hepatitis and bacteriophages targeting this bacterium decreased intrahepatic levels of its major toxin cytolysin and improved ethanol-induced liver disease in mice.

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The Sookoian study opens potentially a new avenue in hepatology. Many questions arise: are detected bacterial DNA fragments biologically relevant? How might those bacterial fragments affect immunity and inflammatory processes? Can whole bacteria be cultivated from a diseased liver? Where are the bacteria located if any: intracellular or extracellular and in which cell types? Can some specific bacterial DNA trigger the next steps towards aggravated liver diseases? The field of hepatology has the privilege to have access to various tissues including intestine, liver and also portal vein blood. Therefore, hepatologists together with microbiologists and immunologists have the chance to learn how relevant intrahepatic bacterial components are in the pathophysiology of early and advanced liver diseases. Further studies assessing in parallel various liver pathologies and gut/liver microbiome are eagerly awaited.

Contributors HT, RB and VT wrote the article.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Tilg H, Burcelin R, Tremaroli V. *Gut* 2020;**69**:1373–1374.

Received 26 January 2020
Revised 6 February 2020
Accepted 6 February 2020
Published Online First 14 February 2020

► http://dx.doi.org/10.1136/gutjnl-2019-318811

Gut 2020;**69**:1373–1374.
doi:10.1136/gutjnl-2019-320490

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