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Gut-liver axis beyond the microbiome: How the fungal mycobiome contributes to alcoholic liver disease.

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Commentary on "Intestinal fungi contribute to development of alcoholic liver disease" Yang et al, J Clin Invest 127:2829-2841 (2017)

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The importance of the "Gut-Liver Axis" has been long established in alcoholic liver disease. The direct effects of alcohol, its metabolites and reactive oxygen species produced during alcohol metabolism result in cellular stress in hepatocytes, release of damage-associated molecules (DAMPs) and increased hepatocyte vulnerability to inflammation-related cellular injury. Excessive alcohol use also results in gut "leakiness" resulting in increased delivery of pathogen-derived molecular patterns (PAMPs) to the liver via the portal system. All of these gut-derived PAMPs and hepatocyte-derived DAMPs contribute to Kupffer cell and innate immune cell activation in the liver in alcoholic liver disease (1). Alcohol use was shown to change the composition of the gut microbiome by modifying the quantity, quality and diversity of bacteria in the intestines both in humans and mice (1). The composition of the gut microbiome is only partially understood and it includes bacteria, fungi and viruses. Every individual's gastrointestinal tract contains thousands of different species of microbes of which 99.9% belong to only a few species. The less abundant component of the microbiome is termed as "rare biosphere" that is more diverse and appears to have a major impact on health and disease. The fungal microbiota, also referred to as "the mycobiome", is part of the rare biosphere and this is a new and rapidly emerging field on which scientific knowledge lags behind that of the bacterial microbiome. Increasing evidence suggests that the fungal microbiome plays a role as a co-factor in inflammatory and metabolic disorders and also in modulating the bacterial microbiome and host defense. The fungal microbiome has been studied at mucosal sites such as the oral cavity, gastrointestinal and urogenital tracts and the skin. Studies in healthy individuals revealed 66 different fungal genera in the fecal material where the most common genera were Saccharomyces, Candida, and Cladosporium (2). Recent studies indicate correlation between changes in the gut mycobiome and different disease conditions.

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In a recent communication published in the *Journal of Clinical Investigation*, Yang and colleagues demonstrated that chronic alcohol administration increases the mycobiota abundance and causes translocation of fungal β-1,3, glucan into the systemic circulation in mice. Administration of antifungal agents that reduced intestinal fungal overgrowth decreased β-1,3, glucan translocation and ameliorated alcohol-induced liver disease. In the report by Yang et al., fecal samples from patients with alcohol-dependence, alcoholic hepatitis, or alcoholic cirrhosis revealed reduction in fungal species richness and diversity compared to normal controls. Decrease in intestinal fungal diversity has been found previously in inflammatory bowel disease (IBD) (3). A prominent feature of the alcohol-induced fungal dysbiosis was the relative overgrowth of Candida albicans that interestingly was also described as abundant in Crohn's disease. Candida colonization has also been shown to enhance inflammation in the airway (3). In Yang's study, anti-Saccharomyces cerevisiae IgG antibody (ASCA) levels were significantly higher in patients with alcoholic cirrhosis compared to cirrhosis due to chronic HBV infection and ASCA serum levels correlated with mortality in patients with alcoholic cirrhosis. These observations suggest that increased and sustained exposure of the immune system to fungi such as Saccharomyces cerevisiae may contribute to alcohol-related liver cirrhosis, immune dysfunction, or both. The contention that gut-derived fungal PAMPs contribute to ALD is supported by the observation where using bone marrow chimera mice, Yang et al., found that β -1,3, glucan induced liver inflammation via the C-type lectin receptor, CLEC7A (Dectin-1) on Kupffer cells and possibly other bone marrow derived immune cells as indicated by increased IL-1ß production. It should be noted however, that in addition to β -1,3, glucan, many other fungal PAMPs can activate immune cells via various pattern recognition receptors such as toll-like receptors (TLR2,3,4,5,6, and 9), Nod-like receptors (NOD1, NOD2 and NLRP3), C-type lectin receptors (CLRs) and other receptors including CD14, CD36, and galectin 3 (4).

The contribution of the fungal mycobiome to the complexity of disturbed alcohol-induced gut-liver axis has multiple aspects. First, as evidenced by Yang's study, the composition of the mycobiome was changed by chronic alcohol administration in mice as well as in humans with alcoholic cirrhosis. However, the altered intestinal fungal composition does not appear to be specific for ALD as it was found in various other disease conditions including other liver diseases. Nevertheless, abundant evidence suggests that alcohol use changes both the gut microbiome and the mycobiome and further studies may reveal additional details in the micro- and mycobiome that are specific for ALD. It remains to be determined whether the changes in

the microbiome and mycobiome are inter-related and/or the microbiome and mycobiome are equally sensitive to alcohol-induced changes. Additional factors are the host and interactions between the host immune system locally in maintaining the gut integrity, as well as the overall immune homeostasis of the host which are both potentially affected by alcohol use given the immunosuppressive effects of alcohol use. The symbiosis of bacterial and fungal colonization may be another determinant of the healthy versus disease host and may shape gut integrity (5).

The study by Yang et al., demonstrated that soluble β -1,3, glucan activated Kupffer cells and potentially other bone-marrow-derived immune cells via CLEC7A receptors and induced inflammasome activation and IL-1ß secretion. While the authors show that β -1,3, glucan treatment of KCs resulted in cleaved caspase-1 and NLRP3 mRNA upregulation that are indicators of inflammasome activation, it remains unclear whether β -1,3, glucan induced the inflammasome activation alone or another signal(s) were also involved. Previous studies demonstrated that in most cases, inflammasome activation, caspase-1 cleavage and IL-1ß secretion require two signals for functional inflammasome activation and IL-1ß cellular release. Similar to previous reports, Yang et al., showed that the secreted IL-1ß induced by β -1,3, glucan in Kupffer cells results in hepatocyte damage. Indeed, Petrasek et al., has reported inflammasomes activation and the involvement of NLRP3 in alcoholic liver disease and further showed that inhibition of IL-1 signaling and inflammasome activation with IL-1 receptor antagonist has a protective effect in a mouse model of alcoholic hepatitis (6). As a follow-up of this discovery, treatment with anakinra, a recombinant human IL-1Ra, is now under investigation in an ongoing human clinical trial in human alcoholic liver disease (7).

Looking forward, more questions than answers arise when assessing therapeutic implications of the basic research findings on the gut mycobiome in ALD. Although treatment with amphotericin B, a non-absorbable antifungal agent, was effective in mice to prevent alcohol-related liver damage, application of this approach in human disease awaits complex considerations. Interestingly, β-1,3, glucan was suggested to provide health benefits in obesity and metabolic syndrome (8). With the increasing understanding of the health effects of the “gut microbiota” (including bacteria, fungi and viruses), many questions remain unanswered on the interactions between the gut and liver in alcoholic liver disease. It remains to be determined whether the alcohol-related “leaky gut” is solely the result of alcohol and/or its metabolites on the gut mucosa or perhaps the gut microbiota also shapes this relationship. An additional aspect in alcohol use is the direct effect of the ingested alcohol on the intestinal microbiota itself and

potential metabolism of alcohol by the intestinal microbiota. Finally, we need a better understanding of the dynamics and interactions between the various players including bacterial, fungal and viral components in the human "gut microbiota" that shapes health and disease in the host.

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Figure 1: Fungal microbiome contributes to inflammation and hepatocyte damage in alcoholic liver disease

New research shows that chronic alcohol intake changes the gut fungal microbiome and results in increased systemic levels of β-1,3, glucan, a component of Candida. The fungal β-1,3, glucan activates Kupffer cells in the liver to induce interleukin-1ß production that in turn acts on hepatocytes to increase steatosis and promote hepatocyte injury.

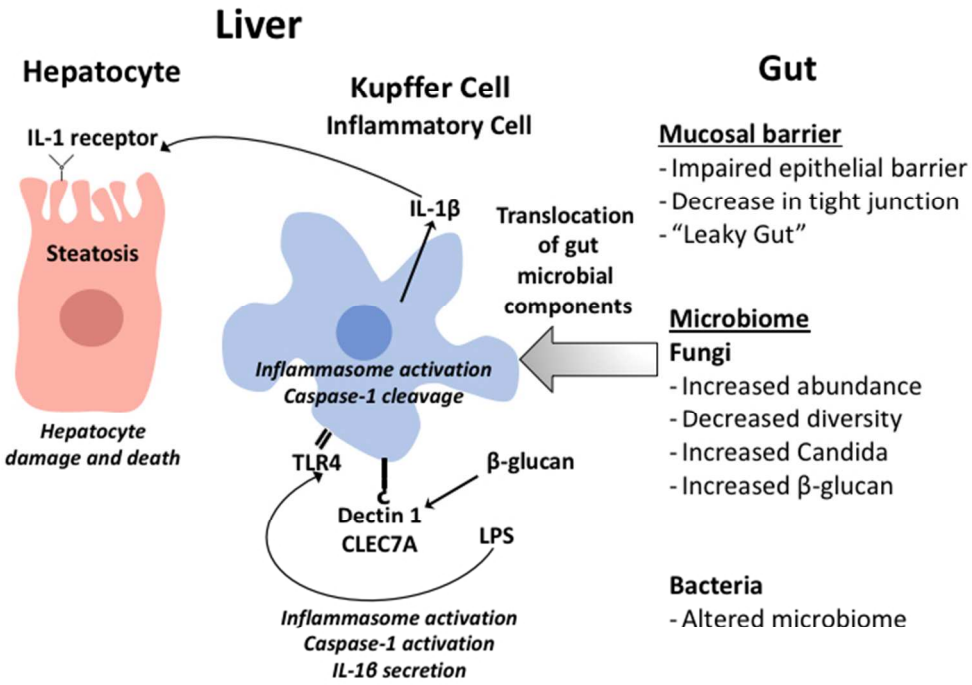

Figure 1

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