

## Host-Microbiome Interactions in Alcoholic Liver Disease

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Alcoholic liver disease is a leading cause of morbidity and liver-related death worldwide. Intestinal bacterial overgrowth and dysbiosis induced by ethanol ingestion play an important role in the pathogenesis of alcoholic liver disease. After exposure to alcohol in the lumen, enteric bacteria alter their metabolism and thereby disturb intestinal homeostasis. Disruption of the mucosal barrier results in the translocation of microbial products that contribute to liver disease by inducing hepatic inflammation. In this review, we will discuss the effects of alcohol on the intestinal microbiome, and in particular, its effects on bacterial metabolism, bacterial translocation and ecological balance. A better understanding of the interactions among alcohol, the host and the microbiome will reveal new targets for therapy and lead to new treatments. (**Gut Liver 2014;8:237-241**)

**Key Words:** Alcoholic liver injury; Microbiota; Permeability; Metabolism

### INTRODUCTION

Alcoholic beverages are widely consumed and are creating an increasing health problem worldwide.<sup>1,2</sup> Because alcoholic liver disease is one of the leading causes for chronic liver disease, recent research focused on elucidating the pathogenesis of alcoholic liver disease. One of the most important factors that contribute to the development of alcoholic liver disease is the gut-liver axis.<sup>3</sup> Gut-derived pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS or endotoxin) translocate from the intestinal lumen to the liver and contribute to hepatic steatosis, inflammation, and fibrosis.<sup>4</sup> The onset of a leaky gut barrier facilitates microbial translocation.

### ALCOHOL-INDUCED CHANGES IN THE GUT MICROFLORA

Alcohol causes intestinal bacterial overgrowth in humans.<sup>5</sup> The number of both anaerobic and aerobic bacteria was significantly higher in jejunal aspirates from patients with chronic alcohol abuse as compared with control subjects.<sup>6</sup> Similar results were obtained in patients with alcoholic cirrhosis.<sup>7</sup> Moreover, the degree of overgrowth correlates with the severity of cirrhosis.<sup>8</sup> Bacterial overgrowth has also been described in experimental animal models of alcoholic liver disease. After 3 weeks of intragastric alcohol feeding, mice with alcoholic liver injury showed higher aerobic and anaerobic bacteria almost along the entire gastrointestinal tract using conventional culture techniques.<sup>9</sup> Culture-independent amplification of the 16S ribosomal RNA by quantitative PCR (qPCR) confirmed an increase of the total amount of enteric bacteria in the cecum of alcohol-fed mice as compared with isocaloric diet fed mice.<sup>9,10</sup>

Chronic alcohol ingestion not only results in quantitative changes of the intestinal microflora, but it also leads to enteric dysbiosis.<sup>10,11</sup> Dysbiosis is known as a condition in which the microflora becomes unbalanced and the symbiotic relationship between the host and microbiome is lost. We reported that intragastric feeding of alcohol for 3 weeks is associated with a relative abundance of Bacteroidetes in the cecum, while control mice showed a relative predominance of Firmicutes.<sup>9</sup> In particular, commensal probiotics including *Lactobacillus* (belonging to the phylum Firmicutes) are relatively lower after chronic alcohol administration. We have confirmed a lower abundance of *Lactobacillus* in the alcohol group compared with isocaloric diet fed control mice using the Tsukamoto-French intragastric feeding model or the Lieber-DeCarli model with qPCR (unpublished data).<sup>10</sup> Rats treated with daily alcohol gavage for 10 weeks exhibit altered mucosa-associated microbiota composition in the colon.<sup>12</sup> The mucosa-associated bacterial taxonomy was evaluated in patients with alcoholic cirrhosis or in alcoholics without

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liver disease using 16S rRNA sequencing. The abundance of Bacteroidaceae was lower in the alcoholic groups as compared to healthy individuals.<sup>13</sup> Moreover, patients with alcoholic liver cirrhosis showed a significant increase in the family of *Prevotellaceae* in the feces compared with hepatitis B related cirrhosis or healthy controls using sequencing of the common 16S rRNA region of bacteria (Table 1).<sup>14</sup> Dysbiosis together with intestinal bacterial overgrowth increases luminal levels of microbial products. We will discuss in the following how alcohol-associated dysbiosis might either directly or indirectly affect the pathogenesis of alcoholic liver disease.

### MICROBIAL TRANSLOCATION ASSOCIATED WITH ALCOHOLIC LIVER DISEASE

Translocation of microbial products results from an increased intestinal permeability, which is very important in the pathogenesis of alcoholic liver disease.<sup>15</sup> A leaky gut is prevalent in patients with alcohol abuse. Using chromium-51-EDTA (edetic acid) absorption test, alcoholic patients showed higher permeability of the small intestine than controls.<sup>16</sup> Even a single oral dose of ethanol increases gastroduodenal permeability.<sup>17</sup> Alcoholics with chronic liver disease showed a marked and highly significant increase in both lactulose absorption and in the urinary lactulose/mannitol ratio after oral administration of lactulose and mannitol demonstrating enhanced intestinal permeability.<sup>18</sup> Another interesting clinic study showed that patients with alcoholic liver disease did not exhibit higher intestinal permeability to polyethylene glycol M, 400. However, intestinal

absorption and urinary concentrations of polyethylene glycol M, 1500 and M, 4000 were significantly increased in patients with alcoholic liver disease.<sup>19</sup> An increase in intestinal permeability is also observed in experimental animal models of alcoholic liver disease. Acute ethanol administration results in increased FITC-dextran permeability in mice.<sup>20</sup> Chronic alcohol ingestion resulted in elevated fecal albumin concentrations suggestive of gut leakiness.<sup>10</sup>

Increased intestinal permeability can result from disruption of tight junction proteins. Ethanol alone is able to disrupt tight junction function *in vitro*.<sup>21</sup> However, acetaldehyde as the oxidative metabolite of ethanol is believed to play a more important role in tight junction regulation during alcoholic liver disease. Acetaldehyde redistributes tight junction proteins and increases tight junction protein phosphorylation.<sup>22,23</sup> Treatment of polarized Caco-2 cells with acetaldehyde is a well-established *in vitro* model to mimic intestinal barrier dysfunction during ethanol exposure *in vivo*.<sup>24</sup> Besides ethanol and acetaldehyde as its metabolite, intestinal microbiota itself might trigger tight junction dysfunction by inducing intestinal inflammation. Such a concept has been proposed for the progression of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis. The loss of NLRP3 and NLRP6 inflammasomes in mice is associated with intestinal dysbiosis and results in inflammation of the colon via the chemokine CCL5. A subsequent onset of a leaky gut induces translocation of bacterial products to cause liver disease progression.<sup>25</sup> Subclinical intestinal inflammation in the lamina propria could play a similar role in alcoholic liver disease. A mast cell membrane stabilizer prevents ethanol-induced epithelial barrier alteration in an animal model of alcoholic disease.<sup>26</sup> Therefore, alcoholic-induced dysbiosis might induce intestinal inflammation resulting in a disruption of the intestinal epithelial cell integrity and bacterial translocation.

LPS or endotoxin is a well known bacterial product that is elevated in plasma of both alcoholic patients and experimental animal models of alcoholic liver disease.<sup>27,28</sup> Luminal LPS can translocate and reach the liver via the portal vein to activate Kupffer cells via Toll-like receptor (TLR)-4 and/or CD14 signaling and to induce proinflammatory cytokine production.<sup>29</sup> Mice deficient in the LPS receptor TLR4 or co-receptor CD14 are resistant to alcohol induced liver disease.<sup>30,31</sup> Notably, plasma endotoxin levels were not different between TLR4 deficient and wild type mice<sup>30</sup> indicating that the TLR4 signaling pathway does not mediate gut leakiness. LPS binding protein (LBP) is reported to promote alcohol-induced liver injury through enhancing LPS-induced signal transduction in mice.<sup>32</sup> In addition to LPS, other bacterial products such as bacterial DNA could also translocate from the intestinal lumen to extraintestinal space and organs. Bacterial DNA is elevated in the plasma of patients with alcohol related cirrhosis.<sup>33</sup> Bacterial DNA is recognized by TLR9 and could sensitize the liver to injury induced by LPS.<sup>34</sup> Taken together, a leaky gut enables bacterial products to trans-

**Table 1.** Changes in the Intestinal Microbiota Associated with Alcoholic Liver Disease

Study level	Implicated microbiota	Methodology	Ref.
Mouse model	In alcohol-fed mice:	16S rRNA gene	9
(Tsukamoto-	<i>Akkermansia</i> ↑	pyrosequencing	
French intragas-	<i>Bacteroidales</i> ↑	Cecum samples	
tric model)	<i>Lactococcus</i> ↓		
	<i>Lactobacillus</i> ↓		
	<i>Leuconostoc</i> ↓		
	<i>Pediococcus</i> ↓		
	<i>Lactobacillales</i> ↓		
	<i>Lactobacillaceae</i> ↓		
Human	In alcoholics:	16S rRNA gene	13
(alcoholics)	<i>Bacteroidaceae</i> ↓	pyrosequencing	
		Recto-sigmoid	
		biopsies	
Human	In alcoholic cirrhosis:	16S rRNA gene	14
(liver cirrhosis	<i>Prevotellaceae</i> ↑	pyrosequencing	
secondary to		Stool sample	
alcohol abuse)			

locate from the intestinal lumen to the liver, where they are major contributors to alcoholic liver disease.

## EFFECT OF ALCOHOL ON METABOLISM OF THE INTESTINAL MICROFLORA

Ethanol can be metabolized not only by the liver but also by the intestinal microbiota in the gastrointestinal tract. Some bacteria like *Escherichia coli* oxidize ethanol into acetaldehyde under aerobic, microaerobic, and anaerobic conditions via an alcohol dehydrogenase dependent reaction.<sup>35</sup> Additionally, other pathways such as catalase metabolize ethanol in human colonic bacteria.<sup>36</sup> Moreover, germ-free rats showed less acetaldehyde content than conventional animals in both rectum and cecum after ethanol treatment.<sup>37</sup> Interestingly, the activity of aldehyde dehydrogenase is very low in colonic mucosa, which limits the transformation from acetaldehyde into acetate.<sup>38</sup> This will further lead to accumulation of acetaldehyde in the intestinal lumen. Taken together, intestinal bacteria metabolize alcohol and increase luminal acetaldehyde concentrations. As discussed above, acetaldehyde disrupts the mucosal barrier in the intestine suggesting a direct effect of the gut microbiota on microbial translocation. Whether an alcohol-associated dysbiotic microbiome has a greater capacity to metabolize alcohol and to generate acetaldehyde requires further investigations.

In addition to the metabolism of ethanol by the microflora, alcohol could also affect bacterial metabolism and alter metabolites in intestinal bacteria. However, there are few reports that are directly addressing this possibility. To date the most comprehensive study employed high performance liquid chromatography time-of-flight mass spectrometry and gas chromatography-mass spectrometry analytic methods to characterize metabolic alterations in luminal contents of the gastrointestinal tract following chronic ethanol administration in rats. Metabolites that are affected by ethanol feeding include amino acids and derivatives, steroids and derivatives, fatty acids and conjugates as compared with control rats.<sup>39</sup> The relevance of these changes for alcoholic disease progression remains to be determined in

future studies.

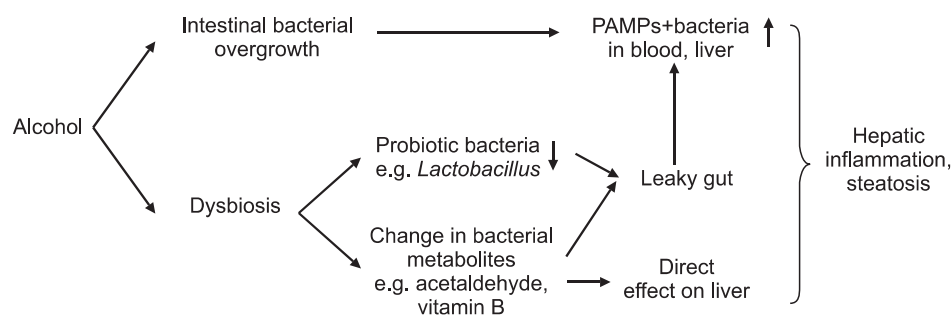
Vitamin metabolism is another interesting aspect that is affected during chronic alcohol consumption. Chronic alcoholics are deficient in water soluble vitamins, particularly in vitamin B family members. Thiamine (vitamin B<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>), pyridoxine (vitamin B<sub>6</sub>), folate (vitamin B<sub>9</sub>), and biotin (vitamin H) deficiencies are commonly found in patients with chronic alcohol abuse.<sup>40-42</sup> Chronic alcohol feeding inhibits carrier-mediated intestinal vitamin uptake, which is often associated with a decrease in the expression of the respective vitamin carrier expression.<sup>42</sup> The intestinal microbiota synthesizes multiple vitamin B family members and is a major source of vitamin B *in vivo*.<sup>43</sup> Enteric dysbiosis is associated with vitamin B deficiency.<sup>44</sup> Whether alcohol inhibits vitamin biosynthesis in intestinal bacteria and contributes to deficiencies seen in chronic alcoholics requires further investigation.

## CONCLUSIONS

Intestinal bacterial overgrowth and dysbiosis are prominent changes that occur in the microbiome during chronic alcohol consumption. At the same time, the intestinal mucosal barrier becomes leaky and allows bacterial products to translocate to the liver, where they can cause disease progression. The impact of alcohol on intestinal microbiome during alcoholic liver disease development has been summarized in Fig. 1. How exactly changes in the gut microflora contribute to the onset of a barrier dysfunction and to a disturbance of intestinal homeostasis warrants further investigation. Understanding the detailed effect of ethanol on the intestinal microbiome and bacterial metabolism, and how such effects promote alcoholic liver disease progression, will not only provide novel insights into the pathogenesis of alcoholic liver disease but will also reveal new therapeutic targets for treatment of patients with alcoholic liver disease.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was



**Fig. 1.** The effects of alcohol on the intestinal microbiome during the development of alcoholic liver disease. Alcohol ingestion causes intestinal bacterial overgrowth, alterations in microbiota composition and changes in the intestinal metabolome. These effects, in turn, lead to increased bacterial translocation and result in alcoholic liver disease. PAMPs, pathogen associated molecular patterns.

reported.

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