Editorial

Gut microbiome and intestinal barrier failure – The "Achilles heel" in hepatology?

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Bacterial translocation (BT) includes the migration of viable micro-organisms but also all microbial products (endotoxins such as lipopolysaccharide [LPS], lipoteichoic acid, bacterial DNA, peptidoglycans, and fragments, e.g. muramyldipeptide, etc.) across an even, anatomically intact intestinal barrier from the intestinal lumen to mesenteric lymph nodes (MLN) and other extraintestinal organs and sites [2]. Although the term "BT" summarizes all these various bacterial products, the route, and site as well as the immunological response between them are most likely very different. BT in liver disease has been extensively studied in the past, mainly due to its long known role as an underlying mechanism in the development of spontaneous bacterial infections such as spontaneous bacterial peritonitis (SBP) in decompensated cirrhosis [17]. Moreover, in advanced cirrhosis, pathological BT most likely impacts on the natural course of liver cirrhosis via triggering and/or aggravating hepatic failure, encephalopathy or hepatorenal syndrome. On this background, it has been proposed that intestinal decontamination could improve disease severity in cirrhotic patients [7]. Concerning the mechanisms promoting BT in chronic liver disease, the majority of the studies have been performed in decompensated cirrhosis unraveling three main factors: intestinal bacterial overgrowth (IBO), increased intestinal permeability (IP), and impaired immunity. IBO has usually been evaluated at only one site, namely the upper small intestine, by applying culture techniques since it is defined as >10^5 CFU/ml aspirate. However, it needs to be stressed that only a minority of the enteral flora can be cultured by conventional techniques and whether the small intestine is the site of most prevalent translocation has not been addressed so far. Nonetheless, in advanced liver cirrhosis IBO is a very frequent finding and has been linked to the development of BT, endotoxemia, and SBP [1,12]. Indeed, bacteria causing SBP are not only normal commensal gut bacteria but most frequently exactly those overgrowing in the small intestine. So it has been said that BT is caused by "too much of the good guys at the wrong place" since, the upper intestine is not made to host this load of bacteria. Increases in IP in cirrhosis have been reported by various methods and factors involved including structural changes, oxidative stress, and alterations in enterocyte mitochondrial function [17]. Moreover, the secretory barrier limiting adhesion of bacteria to the epithelium is diminished due to deficiencies in bile, IgA, and antimicrobial peptides [6,9,16].

More recently, BT has been recognized as crucial event also in non-decompensated and non-cirrhotic stages of liver disease being particularly relevant for the progression of liver disease, i.e. fibrogenesis [5,14], inflammation [15], and –possibly– cancerogenesis [19]. However, so far the mechanisms leading to pathological BT in these early phases of liver disease have not been addressed. In this issue of the *Journal of Hepatology,* Fouts *et al.* applied (i) DNA pyrosequencing techniques enabling differentiated characterization of the intestinal microbiome; (ii) an intestinal loop model for evaluation of intestinal permeability in various segments along the intestinal tract (iii) at various time-points during the early course of two different animal models of liver injury [4]. BT occured independently of any microbial changes both quantitative and qualitative in bile-duct-ligated (BDL) rats and preceded the occurrence of bacterial overgrowth in CCl_4-induced liver injury. Moreover, increased intestinal permeability throughout the intestine (except for proximal small bowel) was observed as early as one day following liver injury in both models and was associated with an early increase of systemic LPS levels as well as the number of viable bacteria in MLN. This provocative original investigation sheds light on the sequence of events after acute liver injury culminating in BT. Indeed, these data in conjunction with previous data by the same group [18] underscore the primary role of barrier failure independent from any alteration in intestinal flora, easing the access of bacteria and bacterial products from the gut to the circulation in liver injury. It cannot be assumed that observations made in cirrhotic conditions are transferable to the scenario of early liver disease. In fact, advanced cirrhosis is characterized by marked hemodynamic, metabolic, and immunological alterations secondary to liver failure and portal hypertension, that are absent in early stages of the disease. Therefore, unraveling the mechanisms causing the intestinal barrier to fail in early phases of liver injury seems of upmost relevance for the "hepatologist" of the future, expanding the focus clearly beyond the liver towards the gut and its habitants.

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In agreement with the essential role of occludin for intestinal integrity and tight junction function, Fouts *et al.* observed decreased expression of occludin in both animal models [4]. However, most tantalizing is the fact that occludin is downregulated at different sites with reduced expression in colon and small intestine in BDL- and CCl4-mice, respectively. However, increases in IP were observed also at sites with normal occludin expression emphasizing the complexity of IP regulation. Indeed, occludin represents only one out of more than 50 tight junction proteins and TJ-function is not only a matter of protein expression. Intestinal integrity and permeability are known to involve Rho GTPases, myosin light chain kinase, and various non-epithelial cell types such as intraepithelial lymphocytes [10].

Likewise, highly interesting in Fouts *et al.* investigation is the finding that LPS levels did not decrease despite a greater than 90% reduction of intestinal flora by treatment of mice with non-absorbable antibiotics [4]. This may hint to the vast importance of mucosa-adherent *vs.* luminal (free) bacteria. The former is clearly the site of major interest since it is potentially less accessible for antibiotic treatment but via attachment to the epithelium and transcytosis is the one that is translocating. In that regard, it still remains to be clarified why only a real minority of bacterial species translocate to MLN in liver disease. Also these new data by Fouts *et al.* again find *Enterobacteriaceae* among the most frequent species translocating. This translocation occurs independently of the LPS/TLR4 signaling pathway in BDL rats. However, commensal bacteria are composed of a number of molecular structures activating multiple TLRs and besides TLRs other pattern-recognition receptors (PRR) such as membrane-bound C-type lectin receptors or NOD-like-receptors play a central role in the initiation of immune response to translocating bacteria [8]. Most likely, various PRRs in concert orchestrate this scenario and thus, delineating the relative contribution of each of these PRRs will represent the "Gordian knot" to elucidate the phenomenon of pathological BT in liver disease.

The study by Fouts *et al.* also clearly expands our understanding of how different models of liver disease impact on the gut microbiome by showing that BDL within days led to bacterial overgrowth of aerobic and anaerobic germs in the small and large intestine whereas in CCl4-induced liver injury quantitative changes of intestinal bacteria occurred only after 8 weeks of treatment when fibrosis had already developed. The bacterial spectrum (namely microbial species richness and evenness) remained unaltered in BDL but a dysbiosis was observed in CCl4-treated mice. This dysbiosis reflects an increased number of operational taxonomic units within the phylum *Firmicutes*, (e.g. *Lactobacillus*) and *Actinobacteria* along with a marked reduction in microbial diversity. Reduced microbial diversity has also been observed in cirrhotic patients [3], and otherwise has been associated with obesity and inflammatory bowel disease [11]. Finally, pyrosequencing analyses in animals treated with CCl4, alcohol feeding, BDL or a genetic fatty liver model, revealed that the enteric microbiome differs with respect to the etiology of liver disease and shares only some operational taxonomic units. Thus, it appears not conclusive that a unique microbial species is of crucial relevance for progression of liver disease independent of its etiology.

However, a number of intriguing questions remain. First, is it liver injury *per se* that disrupts the homeostasis of the gut microbiota or is the causative agent (e.g. alcohol, CCl4) or stimulus (e.g. diet) or missing bile acids (e.g. in BDL) causing the composition and density of the intestinal flora to change? Utilizing pure intrahepatic models of liver injury such as viral hepatitis should provide insight into this question. Second, what measures are most suited to lower inflow of LPS (and other bacterial products and/or bacteria) in liver disease? This "billion dollar question" mainly relates to potential detrimental side effects known to be associated with long-term use of antibiotics and underlines the need for a more detailed understanding of the host-microbial homeostasis at the intestinal mucosal surface during liver disease. Indeed, recent data in HBV and HCV patients indicate that host factors (e.g. intestinal integrity and host response to bacterial products) are more important than changes of the microbiome for the progression of liver disease [13]. Thus, improving barrier function and limiting the host response to BT may be the key to success.

At the end it may clearly be important to know "who lives" and "at which site" in the intestinal tract of an individual at risk for or with ongoing liver injury. Advancing our knowledge of the gut microbiome and of the mechanisms altering intestinal barrier function in early stages of liver injury and disease will help to more accurately target this "Achilles heel in liver disease" and therefore, will guide the development of novel therapeutic and particularly preventive strategies.

Conflict of interest

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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