The Great Escape: Microbiotal LPS Takes a Toll on the Liver

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Abstract

The interaction between the intestinal microbiota and host is much more complex than previously appreciated, and we are now learning that it can have an impact on extraintestinal human diseases. In this issue of the journal (beginning on page 1090), Lin and colleagues present important data linking the microbiota, lipopolysaccharide (LPS), and toll-like receptor (TLR)4 with hepatitis in a mouse model. These provocative results and those from other recent studies highlight the microbiota as a potential target for therapeutic intervention in several liver diseases. *Cancer Prev Res; 5(9); 1078–80. ©2012 AACR.*

Bacteria that reside in our intestines, collectively called the microbiota, far outnumber our own cells (10^{14} bacteria and 10^{13} total human cells; ref. 1). These bacteria are known to compete with and help prevent infection by intestinal pathogens and some also contribute beneficial metabolic functions. The microbiota has long been thought to be relatively silent in immunologic terms. However, the interaction between the microbiota and the extraintestinal immune system has recently been shown to be much more complex than previously appreciated, and we are now learning that its outcome impacts human physiology in a growing number of disease states including obesity, diabetes, and cancer (2–4).

Because intestinal blood flows through the portal vein to the liver, this organ is the first to come into contact with the intestinal bacteria and/or their inflammatory components (referred to here as microbe-associated molecular patterns; MAMP) that reach the portal circulation. Innate immune cells such as Kupffer cells, hepatic stellate cells, and others equip the liver to respond to bacteria and MAMPs, and this organ detoxifies the blood before it passes to the rest of the body. At least a portion of this response is mediated by the toll-like receptors (TLR) that can recognize MAMPs such as lipopolysaccharide (LPS; using TLR4), bacterial lipoproteins or peptidoglycan (TLR2), or unmethylated CpG DNA (TLR9; 5). These TLRs signal through the adaptor protein MyD88 to initiate a signaling cascade that leads to the activation of transcription factors, including NF-κB, ultimately inducing the production of proinflammatory cytokines (e.g., IL-6), chemokines, and other proteins involved in host defense. Interestingly, higher levels of bacteria/ MAMPs translocate from the intestine and into the portal

circulation in patients with liver diseases such as cirrhosis as compared with healthy individuals (6), where these MAMPs may induce increased TLR signaling. However, the effect of increased bacteria/MAMP translocation and innate immune recognition on disease in the liver is not clear.

Lin and colleagues now make an important contribution to our understanding of the pathologic consequences of the interaction between the intestinal microbiota and the innate immune system by showing that these bacteria and/or their LPS can be recognized by TLR4, promoting hepatitis (7). Using the lectin concanavalin A (ConA) to induce hepatitis, the authors show that treatment with a nonabsorbable cocktail of broad-spectrum antibiotics (used to specifically eradicate or reduce intestinal bacteria) significantly decreased liver damage as measured by histology, levels of the liver enzyme alanine aminotransferase (ALT) in the blood, and hepatic cell death. This correlated with decreased levels of proinflammatory cytokines in the liver. These intriguing data show that, in at least one model of acute hepatitis, the microbiota promotes the inflammatory response in the liver. Furthermore, the authors show that TLR4-deficient mice exhibit less liver damage and inflammation than wild-type mice, implicating LPS from the microbiota as a major contributor to the inflammatory signaling observed. To further highlight the ability of LPS to promote these responses, the authors demonstrate that purified LPS can potentiate liver pathology in the ConA model. In addition, concomitant with the increased inflammatory response after ConA treatment, the authors show a limited but significant increase in T-cell recruitment and activation. Finally, by conducting adoptive transfers with wild-type or TLR4$^{-/-}$ splenocytes, containing T cells and other immune cells, the authors show that TLR4 on immune cells is sufficient to cause liver pathology. These findings provide an important addition to a growing body of literature implicating the microbiota as an important factor in liver diseases.

The present work by Lin and colleagues builds upon and extends earlier research that used a diverse set of models and implicated the TLR4/MyD88/NF-κB axis in liver disease. Forced expression of the NF-κB inhibitor, IκB, was shown to attenuate disease progression in the Mdr2 knockout mouse

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model of hepatocellular carcinoma (HCC; ref. 8). Genetic deletion of IKKβ in myeloid cells and hepatocytes, a signaling protein upstream of NF-κB, attenuated disease in a chemical model using diethylnitrosamine (DEN) to induce liver damage and HCC (9). Two studies have shown a role for MyD88 in liver disease; Ojiro and colleagues using the ConA hepatitis model (10) and Naugler and colleagues who showed that deficiency in MyD88 led to a decrease in HCC in the DEN model (11). Furthermore, Naugler and colleagues linked disease to production of the downstream proinflammatory cytokine IL-6, that was essential for HCC (11). This supports the current data from Lin and colleagues, who show that TLR4 is critical for IL-6 production in the ConA model of hepatitis (7). Together, these studies identified the TLR4/MyD88/NF-κB axis as playing a role in specific liver diseases/conditions, but did not study the source of the ligands that activated this pathway. This link was first shown by Seki and colleagues, who used a bile duct ligation model of liver injury to show that the microbiota signaling through TLR4 and MyD88 promote liver inflammation and fibrosis (12). Dapito and colleagues showed that the microbiota promote HCC through activation of TLR4 using a DEN/CCl₄ model (13); although in this latter study, the investigators actually found slightly increased liver injury in the TLR4-deficient mice, reflecting the complexity of outcomes depending on the context in which this signaling occurs. Together, these studies elegantly show the role of the microbiota and the TLR4/MyD88/NF-κB axis in models of liver disease (7, 12, 13).

Interestingly, the link between TLR4 and liver disease is supported by genetic data from humans. A TLR4 polymorphism was identified as part of a genetic signature that is predictive of decreased risk of development of cirrhosis (14). This polymorphism results in a compromised TLR4 that is attenuated for signaling in response to LPS (15). Further supporting the link between LPS, TLR4, and liver disease in humans, is evidence that patients with cirrhosis have higher levels of LPS in their blood (16). Together, data from clinical studies and animal models strongly indicate that recognition of microbiotal LPS by TLR4 contributes to diverse liver diseases.

Because TLR4 can promote liver disease, an interesting question is whether other innate immune receptors, including other TLRs, are involved as well. Bacteria contain numerous MAMPs, so it is logical that other ligands, in addition to LPS, could contribute to liver inflammation and pathology. In fact, using the bile duct ligation model, Miura and colleagues showed a critical role for TLR9 in liver fibrosis (17). In this context, TLR9 presumably signals in response to bacterial DNA derived from the intestinal microbiota. Using the same model, another study found no role for TLR2 in the fibrotic response (12). Interestingly, TLR3 has been shown to be involved in promoting hepatitis using the ConA model, although no link to the microbiota was shown in this study (18). TLR3 can respond to double-stranded RNA from viruses, but the authors suggest that TLR3 may be activated in response to the release of nucleic acids from damaged or dying liver cells in this model (18).

In addition to TLRs, many other innate receptors recognize microbes and play a role in inflammatory responses. NLRs (Nod-like receptors) initiate the activation of cytosolic protein complexes called inflammasomes that lead to the secretion of the proinflammatory cytokines IL-1β and IL-18 (19). These cytosolic receptors can play a role in intestinal inflammatory conditions such as colitis (20), and it will be interesting to determine whether they are involved in the response of the liver to translocated bacteria/MAMPs and subsequent liver diseases.

Another important question is which factors lead to increased intestinal permeability and bacteria/MAMP translocation, as these are the initial events that subsequently lead to TLR signaling and accelerated inflammation in the liver. This phenomenon may be linked to decreased intestinal motility, intestinal bacterial overgrowth, mucosal oxidative stress, intestinal inflammation, portal hypertension, and/or compromised tight junctions, and further studies will be required to evaluate the relative contributions of these diverse factors (6). A greater understanding of the molecular mechanisms that lead to altered permeability will hopefully facilitate therapeutic interventions. Proteins whose activity may promote increased intestinal permeability include JAM-A (21) and the nonmuscle form of myosin light chain kinase (MLCK; ref. 22). LPS-induced intestinal permeability was inhibited by an MLCK inhibitor in rats (23). Furthermore, transgenic mice expressing constitutively active MLCK in intestinal epithelial cells exhibit tight junction dysfunction and increased basal immune activation (22). It may be instructive to use this genetic model to examine the role of increased bacteria/MAMP translocation in liver disease and study whether intestinal barrier dysfunction alone predisposes mice to such diseases.

Because many liver diseases develop chronically, it is important to understand at what stage translocated bacteria/MAMPs and innate signaling play a role. HCC, like other cancers, depends on an initiating mutational event as well as potentiating events that lead to progression. Pikarsky and colleagues, using the Mdr2 model, found that NF-κB promoted HCC progression, but not its initiation (8). In agreement, Dapito and colleagues showed elegantly using the DEN/CCl₄ model that the microbiota and TLR4 contribute specifically to the progression of HCC (13). In fact, by using antibiotics to eliminate the microbiota, the authors show that progression to HCC can be inhibited by as much as 90%. Collectively, these remarkable results highlight the fact that therapeutics targeting the microbiota or preventing bacteria/MAMP translocation or inflammatory signaling may be able to prevent the progression of some cancers before they have a significant effect on human health.

The prospect of stifling some cancers by eliminating the microbiota is exciting, but this approach would likely have deleterious long-term health consequences. Similarly, drugs that inhibit TLR4 or other innate immune signaling components may have beneficial effects on certain liver diseases, but it is not clear whether they would be tolerated long-term as they would likely have a negative impact on innate

immune function and defense against infection. Perhaps the most benign yet potentially effective strategy would be to alter the makeup of the intestinal microbiota. Recent studies suggest that the microbiota contains both beneficial and potentially harmful bacteria and that an incorrectly balanced microbiota can contribute to disease (6). As this exciting area is explored in greater detail, we will learn which specific bacteria are beneficial or detrimental and through the use of probiotics or dietary changes (which can alter the microbiota), may be able to harness this information to reverse the chronic low-grade inflammatory state thought to occur in the diseased liver, and thereby prevent the progression to severe diseases such as HCC.

References

1. Fujimura KE, Slusher NA, Cabana MD, Lynch SV. Role of the gut microbiota in defining human health. Expert Rev Anti Infect Ther 2010;8:435–54.
2. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011; 474:327–36.
3. Mathis D, Benoist C. The influence of the microbiota on type-1 diabetes: on the threshold of a leap forward in our understanding. Immunol Rev 2012;245:239–49.
4. Mai V, Draganov PV. Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health. World J Gastroenterol 2009;15:81–5.
5. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010;11:373–84.
6. Almeida J, Galhenage S, Yu J, Kurtovic J, Riordan SM. Gut flora and bacterial translocation in chronic liver disease. World J Gastroenterol 2006;12:1493–502.
7. Lin Y, Yu L-X, Yan H-X, Yang W, Tang L, Zhang H-L, et al. Gut-derived lipopolysaccharide promotes T cell-mediated hepatitis in mice through toll-like receptor 4. Cancer Prev Res 2012;5:1090–102.
8. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–6.
9. Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–90.
10. Ojiro K, Ebinuma H, Nakamoto N, Wakabayashi K, Mikami Y, Ono Y, et al. MyD88-dependent pathway accelerates the liver damage of concanavalin A-induced hepatitis. Biochem Biophys Res Commun 2010;399:744–9.
11. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007;317:121–4.
12. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324–32.
13. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 2012;21:504–16.
14. Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. Hepatology 2007;46:297–306.
15. Figueroa L, Xiong Y, Song C, Piao W, Vogel SN, Medvedev AE. The Asp299Gly polymorphism alters TLR4 signaling by interfering with recruitment of MyD88 and TRIF. J Immunol 2012;188:4506–15.
16. Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, et al. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. J Hepatol 1995;22:165–72.
17. Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. Gastroenterology 2010;139:323–34 e7.
18. Xiao X, Zhao P, Rodriguez-Pinto D, Qi D, Henegariu O, Alexopoulou L, et al. Inflammatory regulation by TLR3 in acute hepatitis. J Immunol 2009;183:3712–9.
19. Franchi L, Munoz-Planillo R, Nunez G. Sensing and reacting to microbes through the inflammasomes. Nat Immunol 2012;13: 325–32.
20. Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut 2010;59:1192–9.
21. Laukoetter MG, Nava P, Lee WY, Severson EA, Capaldo CT, Babbin BA, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med 2007;204:3067–76.
22. Su L, Shen L, Clayburgh DR, Nalle SC, Sullivan EA, Meddings JB, et al. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. Gastroenterology 2009;136:551–63.
23. Moriez R, Salvador-Cartier C, Theodorou V, Fioramonti J, Eutamene H, Bueno L. Myosin light chain kinase is involved in lipopolysaccharide-induced disruption of colonic epithelial barrier and bacterial translocation in rats. Am J Pathol 2005;167:1071–9.

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