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Silencing a Killer Among Us: Ethanol Impairs Immune Surveillance of Activated Stellate Cells by Natural Killer Cells

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Chronic alcoholic liver disease (ALD) affects millions of individuals worldwide each year. The disease process shares a natural history with other chronic liver diseases and is characterized by early steatosis, inflammation, and necrosis. In some individuals, it will ultimately progress to fibrosis and cirrhosis. Although this progression of chronic liver disease is well characterized, there is no universally accepted therapy available to halt or reverse the process in humans. Instead, clinical treatment focuses predominantly on reducing the effects of decompensation caused by cirrhosis and on transplantation.[1] A “black box” that has added to difficulties in developing treatments for ALD is that, although the risk of ALD increases in a dose- and time-dependent manner with consumption of alcohol,[2-4] only a small proportion of even heavy drinkers develop the severe form of the disease, suggesting the involvement of other factors that modify disease risk.[5] These risk factors may be genetic (eg, polymorphisms in key genes) and/or environmental.

A clear environmental risk factor for developing cirrhosis attributable to alcohol consumption is infection with hepatitis C virus (HCV).[6] Interestingly, this increased risk does not appear to be a simple additive effect of the two hepatotoxins; for example, alcohol does not modify the rate of hepatic injury caused by HCV[7] in spite of the fact that the rate of fibrosis is enhanced greatly.[8-10] Whereas this apparent synergy between alcohol and HCV in hepatic fibrogenesis is well-described, mechanisms underlying the relationship remain unclear. In the study by Jeong et al[11] in this issue, the authors suggest that the synergy between alcohol and HCV may be mediated, at least in part, by impairment of the antifibrotic effects of natural killer (NK) cells.

NK cells are members of the innate immune response and comprise up to 50% of the lymphoid pool in the liver. As with other components of the innate immune response, these cells do not express antigen-specific receptors but rather a series of receptors that serve to distinguish “self ” from “non-self,” or “altered self.” It is well known that NK cells play a key role in the early immune response against virally infected cells, including HCV-infected hepatocytes.[12] In addition to defending against viral infection, NK cells may also directly ameliorate hepatic fibrosis, first by releasing antifibrotic cytokines (interferon [IFN]α and IFNγ),[13,14] and second by killing activated stellate cells (see Jeong et al[11] [Figure 8]).[15] These lines of defense against injury and fibrosis may be compromised in liver disease. It has been shown in clinical studies that NK cell numbers decrease in HCV infection, but that NK cell cytolytic activity is not impaired.[16] Should chronic ethanol exposure inhibit the activity of the depleted pool of NK cells, then the liver's ability to prevent the development of fibrosis would be hampered greatly.

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The experimental design of the study by Jeong et al[11] is relatively straightforward. Animals were exposed to an alcohol liquid diet or isocaloric control diet for 8 weeks, followed by injection with carbon tetrachloride (CCl_4 3 times per week) for 2 weeks. Not surprisingly, ethanol preexposure dramatically enhanced liver damage caused by CCl_4, most likely due to metabolic activation of this toxin by CYPs, especially CYP2E1. The authors therefore titrated down the dose of CCl_4 used in ethanol-exposed mice to match transaminases release observed in the pair-fed controls (0.1 mL/kg vs. 0.25 mL/kg, respectively). Despite similar liver damage at these doses of CCl_4, ethanol preexposure significantly enhanced indices of fibrosis (alpha smooth muscle actin [αSMA] expression, collagen staining, and hydroxyproline levels) and decreased the number of apoptotic stellate cells that were observed.

The authors next showed that poly I:C (an activator of NK cells) did not decrease fibrosis in alcohol-preexposed mice, whereas it effectively decreased fibrosis in pair-fed mice. The subsequent experiments were designed to determine the mechanisms by which this effect of poly I:C is mediated by ethanol. Using a combination of in vivo, ex vivo, and in vitro experiments, the authors show that ethanol impairs the ability of NK cells to kill stellate cells, both by inhibiting activation and release of cytotoxic mediators in NK cells, as well as by blunting apoptosis in hepatic stellate cell (HSCs) in response to these cytotoxic mediators. Unlike in the initial studies investigating the effect of ethanol on CCl_4-induced fibrosis, the authors did not decrease the dose of CCl_4 in the ethanol-exposed group to match transaminases with pair-fed controls, which may have induced artifactual differences between the groups. Nevertheless, these data provide a convincing story indicating that ethanol impairs the ability of NK cells to kill activated stellate cells, which would as a result remove this break in the development of fibrosis. Indeed, the strength of this work is the multiple levels of investigation used to test and validate the authors' underlying hypotheses.

What are less convincing are the experiments, largely co-culture experiments, in which the authors attempt to determine the mechanisms by which NK cells are impaired and stellate cells are injured in the alcohol model. First, the authors demonstrated that ethanol increases transforming growth factor-β (TGFβ) production from stellate cells activated in culture, which can then impair the activation of NK cells. Second, they demonstrate that SOCS1, which inhibits IFNγ-induced HSC cell apoptosis via STAT1 signaling, is increased in stellate cells isolated from ethanol exposed mice. Whereas it is certainly possible that these mechanisms are functional in vivo, these co-culture experiments may miss other mechanisms that are important in vivo. For example, work by Pruett et al[17] has shown that acute ethanol impairs lysis and clearance of B16F10 melanoma cells by NK cells in mice. This effect of acute ethanol is mediated, at least in part, by inducing stress hormones (eg, corticosterone) that decrease both basal and stimulated NK lytic function.[18,19] Although the effect of chronic ethanol on NK function has not been determined previously, it is known that feeding rodents ethanol chronically under conditions similar to those used by Jeong et al[11] will increase plasma corticosterone levels.

There is no ideal rodent model of fibrosis. Thus, the results may be specific to the model used and not the disease it is modeling. For example, transaminase values in both HCV- and ethanol-induced liver disease are often normal or only slightly elevated, unlike the significant elevations observed with CCl_4 administration. The massive hepatocyte death induced in the CCl_4 model may itself stimulate the immune response, and thereby overemphasize the role of immune cells relative to other mechanisms underlying liver disease. This concern is compounded by the fact that the immune system may be altered directly by CCl_4 exposure.[20] The authors' findings might be corroborated in a separate rodent model of fibrosis.

The implications of this work are expansive. First, these results offer rational explanations as to why the rate of fibrosis in HCV patients is accelerated by alcohol consumption. Furthermore,

although it is not possible to test in the CCl_4 model, it is likely that ethanol will also blunt immune surveillance by NK cells against virally infected hepatocytes, which would increase viral load and likely exacerbate the disease. Lastly, the finding that ethanol blocks IFNγ-induced HSC apoptosis suggests that some of the beneficial effects of interferon-based antiviral therapies in HCV-induced fibrosis will be attenuated in the setting of coexisting ALD. The mechanisms identified here also have implications beyond ethanol and HCV. For example NK (and NK T cell) function may be altered in other chronic liver diseases (eg, nonalcoholic fatty liver disease).[21] Furthermore, previous work has demonstrated a correlation between NK cell depletion and the development of HCC in cirrhotics,[22] supporting the role of NK cells in immune surveillance against cancer.[23] Indeed, mouse strains deficient in NK cell products or IFNγ-dependent signaling have high rates of developing spontaneous and induced tumors.[24, 25] The impairment of NK cell functionality and signaling by ethanol may therefore not only enhance the risk of developing cirrhosis, but also the risk of subsequently developing HCC.

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