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Neurologic Cues Modulate Immune-Mediated Liver Injury and Regeneration

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Hepatic ischemia reperfusion injury occurs during a number of different surgical procedures including liver transplantation and hepatic resection, and during hemorrhagic shock/resuscitation and other hypotensive events. While limited cell death may occur during the ischemic phase, especially when ischemia is kept to shorter periods, tissue reperfusion activates an inflammatory cascade that dramatically exacerbates liver injury.¹ This is largely mediated by resident Kupffer cells and recruited neutrophils, which produce cytotoxic reactive oxygen species that post-ischemic hepatocytes cannot effectively detoxify, resulting in considerable cell necrosis.^{1,2} As such, immune-modulatory agents and antioxidants have been proposed as a way to limit hepatic reperfusion injury and improve outcomes during liver transplantation, liver surgery, or during hypoxic hepatitis. However, despite numerous reports of successful therapeutic interventions in animal models, there are, as of yet, no specific and effective therapeutics in clinical use that can inhibit abnormal immune cell activation during hepatic ischemia-reperfusion injury without increasing patient risk for infection.

Netrin-1 is a neuroimmune guidance protein that during development provides chemoattractive and chemorepulsive signals for neuronal cells in the CNS. However, netrin-1 is also expressed in vascular endothelial cells and can regulate inflammatory cell recruitment.³ The importance of netrin-1 has been recognized in a number of inflammatory conditions including ischemia-reperfusion injury in the heart and kidney.⁴ In this issue of *HEPATOLOGY*, Schlegel and colleagues studied the potential role for netrin-1 as a mediator of inflammation in a murine hepatic ischemia-reperfusion model.⁵ The authors first provided evidence for netrin-1 mRNA and protein expression in the liver. Hepatic ischemia (30 min) – reperfusion (3 h) reduced netrin-1 expression. By using a mouse with partial deficiency of the netrin-1 gene (*Ntn1*^{+/-}), the authors were able to further demonstrate that the reduced expression of netrin-1 in the liver exacerbated ischemia-reperfusion injury via enhanced recruitment of neutrophils to the liver. This increased injury correlated with elevated serum levels of pro-inflammatory cytokines (e.g. *TNF-α*) and neutrophil-chemoattractant chemokines such as mKC in *Ntn1*^{+/-} mice.⁵ In a reciprocal experiment, intravenous administration of recombinant netrin-1 protein had the opposite effects; netrin reduced liver injury as well as chemokine production and leukocyte adhesion. Importantly, these experiments demonstrated that netrin-1 only affected the reperfusion injury phase and that it

can be used as a therapeutic agent. Interestingly, these effects appear not to be mediated by the netrin-1 receptor UNC5b, which is expressed on leukocytes,³ but by the A2B adenosine receptor as netrin-1 administration was ineffective in A2BAR^{-/-} mice exposed to hepatic ischemia-reperfusion.⁵ However, it remained unclear why the authors' A2B receptor knockout mice had similar reperfusion injury as the wild type controls, in contrast with previous studies suggesting A2BAR^{-/-} mice had more severe liver pathology after undergoing hepatic ischemia-reperfusion.⁶ The role of UNC5b and the A2B adenosine receptor may need to be investigated in more detail.

Perhaps most importantly, administration of netrin-1 at the time of reperfusion resulted in faster injury resolution and enhanced liver regeneration, likely due to the enhanced production of injury-resolving lipid mediators and increased expression of regenerative markers such as hepatocyte growth factor and vascular endothelial growth factor.⁵ The improved recovery correlated with reduced neutrophil infiltration and improved phagocytosis of dead cells. In support of these findings, Ntn1^{+/-} mice showed the opposite effects, i.e. decreased growth factor expression and reduced PCNA-positive cells. This inhibited pro-regenerative response in Ntn1^{+/-} mice could be reversed by recombinant netrin-1 administration.⁵ Although these findings provided strong evidence that netrin-1 supports tissue recovery after extensive injury, a number of issues need to be investigated. In particular, the cellular sources of endogenous netrin-1 and the actual target cells of both therapeutically administered and endogenously generated netrin-1 remain unclear. Furthermore, the neutrophil-mediated injury phase includes 12–24 h of reperfusion based on both reactive oxygen formation by neutrophils accumulated in the liver⁷ and by release of necrotic biomarkers into the blood.⁸ Treatment of the animals with netrin-1 at the end of ischemia and even at 3 h of reperfusion falls well within the neutrophil-induced injury. Thus, it cannot be excluded based on the experimental design that the improved regeneration is caused at least in part by the effect on pro-inflammatory injury mechanisms rather than by directly stimulating resolution of inflammation and recovery. Addressing this issue could considerably broaden the therapeutic potential of netrin-1 in hepatic ischemia-reperfusion injury and other liver disease models.

Kupffer cells, activated by complement factors, were identified as the primary source of a vascular oxidant stress during the initial phase of hepatic ischemia-reperfusion injury in rodent models.¹ The necrotic cell death and release of damage-associated molecular patterns triggers a second injury phase dominated by neutrophil recruitment into the liver, extravasation and a neutrophil-induced oxidant stress.^{1,2,9} The importance of neutrophil-derived reactive oxygen species such as hypochlorous acid, was shown by the presence of intracellular protein modifications specific for these oxidants.^{7,9} Using antibodies against chlorotyrosine, Schlegel and coworkers provided evidence that in the murine model of hepatic ischemia-reperfusion injury, netrin-1 treatment did not only reduce the number of neutrophils but also attenuated neutrophil cytotoxicity (oxidant stress) after 3 h of reperfusion.⁵ In addition, the authors delineated the subpopulations of macrophages during the early reperfusion phase. Netrin-1 administration resulted in an increase in total F4/80⁺ resident macrophages (Kupffer cells), which was caused by a reduction in CD11b⁺ – cells and a concomitant increase in CD68⁺ – Kupffer cells. Whereas the CD11b⁺ subset of Kupffer cells has a high cytokine producing capacity in response to endotoxin, the CD68⁺

Kupffer cells produce large amounts of reactive oxygen and effectively phagocytose debris.¹⁰ These characteristics of the resident macrophage population correlated with the reduction in cytokine levels and increased phagocytic capacity in netrin-1 treated mice. Overall, this resulted in an attenuated pro-inflammatory milieu and increased neutrophil efferocytosis.⁵ During the late reperfusion phase, the authors also characterized Ly6C expression on newly recruited hepatic monocytes. Classical Ly6C^{high} monocytes (M1) are considered pro-inflammatory due to their high capacity to produce reactive oxygen and generate cytokines such as TNF- α . In contrast, the non-classical Ly6C^{low} monocytes (M2) are considered anti-inflammatory due to their ability to generate cytokines such as IL-10, which down-regulates TNF- α formation, and high phagocytic capacity. Schlegel and colleagues found that netrin-1 treatment decreased the number of classical Ly6C^{high} monocytes and increased the levels of Ly6C^{low} non-classical monocytes, which created an anti-inflammatory and pro-regenerative environment that limited additional injury and supported recovery.⁵ While IL-10 was not measured in this study, it is likely that the higher proportion of Ly6C^{low} compared to Ly6C^{high} monocytes led to increased IL-10 formation, which contributed to the injury resolving nature of netrin-1 administration.

Together these results suggest that neuroimmune guidance proteins such as netrin-1 have a previously unrecognized role in modulating the immune response in clinically relevant models of liver injury. The observations reported by Schlegel and colleagues advances both our understanding of the basic science behind the regulation of the sterile inflammatory response in hepatic ischemia-reperfusion injury and identified a promising novel therapeutic approach.

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