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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Burning, but Not Dying: the Failure of Pyroptotic Cell Death in Hepatocytes

Pyroptosis is a proinflammatory mode of regulated cell death that was first described in macrophages. Pyroptosis is triggered by microbial infection, as well as by a variety of pathogen-associated molecular patterns, such as bacterial lipopolysaccharide (LPS) and nucleic acids, and danger-associated molecular patterns, such as the DNA binding protein high mobility group box-1, oxidized lipoproteins, and host-derived stress signals.[1] Pathogen-associated molecular patterns and danger-associated molecular patterns are sensed by cell-surface pattern recognition receptors, which associate intracellularly to form multiprotein complexes termed *inflammasomes*, which promote the activation of proinflammatory caspase-1 (canonical pathway), and/or caspase-4/-5 (in human beings) and caspase-11 (in mice) (noncanonical pathway). Active inflammatory caspases then cleave the proform of the interleukin 1 (IL1) family of cytokines, including IL1β and IL18, into their active forms. In addition, active inflammatory caspases cleave the cytosolic protein gasdermin D (GSDMD), releasing its N-terminal fragment (GSDMD-N) and allowing its translocation to the plasma membrane, where it oligomerizes to form pores that rapidly result in lytic cell death and release of proinflammatory cytokines. Other gasdermins, such as gasdermin E, have been suggested to execute pyroptosis by similar mechanisms.[2] Given its inflammatory nature, the contribution of pyroptosis to the pathogenesis of liver diseases such as nonalcoholic steatohepatitis, alcoholic liver disease, and viral hepatitis, has attracted considerable interest.[3] However, the occurrence of hepatocyte pyroptosis remains controversial.

In an elegant study featured in the current issue of *Cellular and Molecular Gastroenterology and Hepatology*, Sun et al[4] concluded that hepatocytes are resistant to pyroptotic cell death in vitro and in vivo owing to sublethal caspase-1/11 activation and cleavage of GSDMD observed after exposure to the noncanonical inflammasome inducer LPS and/or the canonical inflammasome inducer nigericin. Sublethal activation refers to activation below the threshold to cause cell death. They then determined that this inability to execute the death program is owing to insufficient expression of caspase-1/11 in hepatocytes, both in physiologic conditions and after priming with LPS. In contrast, liver nonparenchymal cells express high levels of caspase-1/11 and promptly undergo pyroptosis when treated with LPS and/or nigericin. These findings were confirmed in a subsequent series of studies in which the investigators enforced expression of caspase-1 or caspase-11 active domains (p10 and p20) in wild-type mouse hepatocytes in vitro and in vivo, and were indeed successful in inducing rapid pyroptotic cell death. Consistently, hepatocyte pyroptosis was dependent on GSDMD because it was almost undetectable in GSDMD$^{-/-}$ mouse hepatocytes in vitro and in vivo. In wild-type mice, hepatocyte pyroptosis was characterized by GSDMD cleavage; plasma membrane permeabilization accompanied by release of lactate dehydrogenase, alanine aminotransferase, and high mobility group box-1; and organelle dysfunction. These findings provide evidence that the pyroptotic signaling downstream of caspase-1/11 is functional in hepatocytes. However, given the insufficient level of caspase-1/11 expression, it appears unlikely that hepatocyte cell death by pyroptosis plays a major role in inflammatory liver diseases. In fact, low amounts of active caspase-1/11 led to limited GSDMD cleavage, which did not result in significant plasma membrane permeabilization or subsequent lytic cell death Fig. 1. Assuming a few cleaved GSDMD-N fragments still translocate to and insert into the plasma membrane, the inefficient membrane permeabilization could be explained by endosomal sorting complexes required for transport (ESCRT)-dependent repair mechanisms[5] or by the lack of ninjurin 1, a cell-surface protein recently implicated in plasma membrane rupture.[6] Indeed, the investigators found that ninjurin 1 was undetectable in hepatocytes, but highly expressed in macrophages, which are sensitive to pyroptosis.

Contrary to the current results, a recent study by Gaul et al[7] suggested that primary human and mouse hepatocytes exposed to LPS + nigericin showed caspase-1 activation, plasma membrane permeabilization, and cell death. Despite the use of similar inflammasome inducers, the concentrations of both LPS and nigericin were significantly higher than in the current study (10-fold and 2-fold, respectively), which might have resulted in more efficient activation of the inflammatory caspases. Moreover, there were mouse strain differences between the 2 studies, which also may have accounted for the observed discrepancies. Hence, the question still remains whether inflammasome stimuli in vivo during pathophysiological liver insults is sufficient to stimulate hepatocyte pyroptosis. We also note that the higher sensitivity to pyroptosis shown by liver nonparenchymal cells would make them a more likely target than hepatocytes, and could be the source of the increased hepatic GSDMD-N and IL1β, as well as serum active caspase-1 detected in nonalcoholic steatohepatitis patients.[7,8] It also is possible that the sublethal hepatocyte inflammasome activation, GSDMD cleavage, and limited membrane permeabilization may influence the hepatocyte itself and the surrounding nonparenchymal cells in other ways, for example, by providing a more controlled release of proinflammatory and profibrogenic components. Potential mediators may include cytokines (eg, ILIβ, IL18, and so

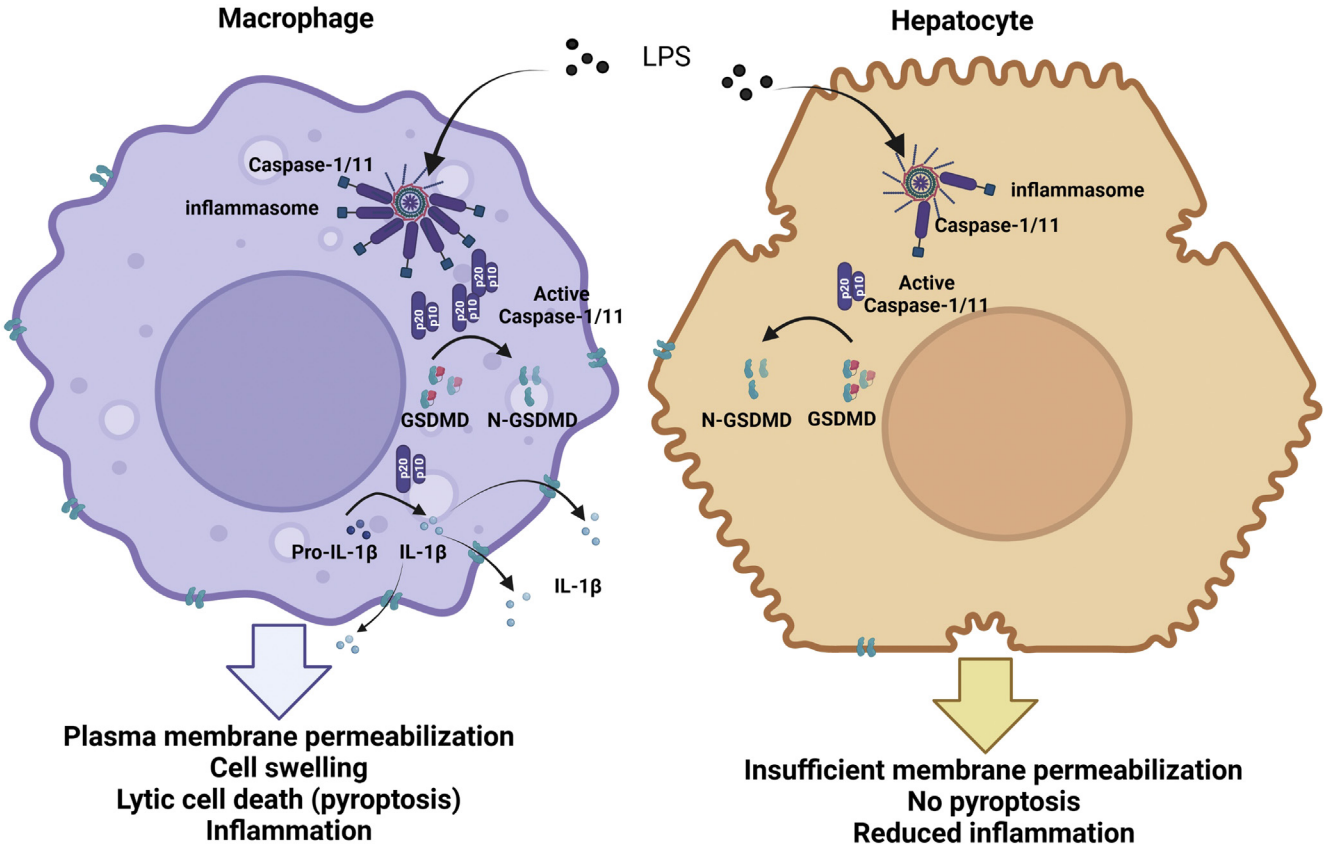

Figure 1. Bacterial LPS stimulates the formation of the inflammasome and activation of the inflammatory caspase-1/11. In macrophages, where expression of caspase-1/11 is high, this leads to substantial GSDMD cleavage and formation of GSDMD-N plasma membrane pores, as well as cleavage and activation of proinflammatory cytokines, such as IL1β, resulting in lytic cell death (pyroptosis) and inflammation. In hepatocytes, where caspase-1/11 expression is low, this process yields insufficient activated caspases to cause membrane permeabilization, therefore avoiding pyroptosis. Image created with Biorender.com. (Toronto, ON, Canada)

forth) and proinflammatory extracellular vesicles, which can activate hepatic stellate cells, macrophages, and perhaps other injurious immune cells in the tissue microenvironment.

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