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Sterile inflammation in acute liver injury: myth or mystery?

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Inflammation during liver injury normally serves as a mechanism for cleaning up debris and as a stimulant for regeneration. However, aberrant levels of inflammation can provoke further liver injury and inhibit regeneration through the release of damaging reactive oxygen species. Considerable effort has gone into understanding the mechanisms that control the switch between healthy and pathological inflammation. The identification of a receptor system that detects damage-associated molecular patterns and stimulates inflammation has led to the idea of sterile inflammation. This article will focus on the role of sterile inflammation during liver injury in three models where sterile inflammation has been presumed to mediate a portion of the injury mechanism and its potential relevance for the human pathophysiology.

Sterile inflammation occurs in the liver during a number of etiologies of liver injury. During hepatocyte necrosis, normal cellular constituents are released from dying cells as damage-associated molecular patterns (DAMPs), and include molecules such as high-mobility group box-1 (HMGB1) protein, ATP, mitochondrial DNA and nuclear DNA fragments that are released from cells and can stimulate toll-like receptors (TLRs) [1]. This leads to the activation of Kupffer cells and other inflammatory cells that express TLRs in the liver, transcriptional activation of cytokine genes and recruitment of cytotoxic cells such as neutrophils and monocytes that can potentially damage hepatocytes. In addition, DAMPs like ATP can activate the Nalp3 inflammasome in Kupffer cells through binding to purinergic receptors resulting in the activation of caspase-1, which processes pro-IL-1β or pro-IL-18 to the active cytokines [1]. Some of the cytokines can stimulate regeneration and recruit more macrophages to clear necrotic cell debris, making space for new hepatocytes. While interventions against sterile inflammation have proven to be highly efficacious in some models, the interpretation of results in other models is still under debate. This editorial will attempt to review the current evidence in favor of, and against, sterile inflammation as a mediator of liver injury, with a focus on clinically relevant models including ischemia–reperfusion injury, acetaminophen (APAP) hepatotoxicity and cholestatic liver injury.

Liver ischemia–reperfusion injury

Liver ischemia–reperfusion injury is an area where sterile inflammation is generally thought to have a well-defined role [2–6]. Ischemia–reperfusion injury occurs in the liver during multiple liver surgeries, but primarily during hepatic resection and liver transplantation. The ischemic period causes cellular swelling and low amount of hepatocyte death due to hypoxia and hyperosmotic swelling [2,4]. Upon reperfusion, the swollen and dead cells release a portion of their intracellular contents, including a number of DAMPs [4]. Extensive complement activation triggers a Kupffer cell–induced oxidant stress, which contributes to the early cell injury [2]. In addition, DAMPs release triggers cytokine formation from local macrophages, which recruits neutrophils that mediate the later portion of the injury [2].

Keywords: acetaminophen hepatotoxicity • damage-associated molecular patterns • ischemia–reperfusion injury • monocytes • neutrophils • obstructive cholestasis

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Inhibition of DAMP receptors such as TLR4 or TLR9, or antagonism of TLRs has repeatedly been shown to protect against hepatic ischemia–reperfusion injury [5,6] by blocking interactions with their ligands such as HMGB1 and DNA, respectively. This results in a reduction in markers of liver injury and a concurrent reduction in recruited neutrophils and activated macrophages. Given the well-defined role of inflammatory cells such as macrophages and neutrophils in the mediation of liver ischemia–reperfusion injury [2] and the efficacy of depletion of either TLRs or their respective ligands, sterile inflammation likely plays a significant role in liver in rodent models of liver ischemia–reperfusion injury and is likely important in human patients undergoing ischemia–reperfusion.

APAP overdose

Whereas there is little controversy in the liver ischemia–reperfusion injury field, there is a substantial debate on whether sterile inflammation exacerbates APAP-induced liver injury. While therapeutic levels of APAP are non-toxic in most cases, an overdose leads to substantial liver injury. It is generally believed that the initial mediator is the reactive metabolite *N*-acetyl-*p*-benzoquinoneimine and its adduction to cellular proteins, which results in mitochondrial oxidant stress and eventual cellular necrosis (reviewed in [7]). The extensive necrotic cell death causes the release of DAMPs including HMGB1, mitochondrial DNA and nuclear DNA fragments. As a consequence, macrophages generate pro-inflammatory mediators, which recruit initially neutrophils and later monocytes into the liver (reviewed in [8]). These events are undisputed during APAP-induced liver injury in mice or humans. However, a debate exists whether this sterile inflammatory response exaggerates the injury via the recruitment of cytotoxic neutrophils or prepares for recovery. For example, it was suggested that IL-1β is an essential pro-inflammatory mediator that promotes APAP-induced liver injury [9]. Although IL-1β is generated through the Nalp3 inflammasome, a protein complex present in immune cells that results in the activation of caspase-1, the absolute IL-1β levels that are being generated are orders of magnitude too low to have an impact on the pathophysiology [10]; in addition, IL-1 receptor-deficient mice are not protected [10]. A number of pharmaceutical interventions that are protective against APAP have also been attributed to a reduction in IL-1β/IL-18 levels via inhibition of the inflammasome (reviewed in [8,10]). However, a majority of studies that suggest a contribution of sterile inflammation to APAP-induced liver injury fail to fully assess metabolic activation of APAP and other off-target effects. For example, the purinergic receptor antagonist A438079 protects against APAP-induced liver injury by inhibiting cytochrome P450 enzyme activities [11] and not by preventing the activation of the Nalp3 inflammasome in Kupffer cells as suggested [12]. Most importantly, neither relevant formation of IL-1β was observed in APAP overdose patients nor were there differences between surviving and non-surviving patients [Woolbright BL, Jaeschke H, Unpublished Data], making it highly unlikely that IL-1β is a critical mediator in the pathophysiology.

All inflammatory cytokines produced during sterile inflammation require neutrophils and/or monocytes to cause toxicity. Another common mistake is to just look at correlations between injury and hepatic neutrophils as evidence for neutrophil-mediated injury. Since sterile inflammation and neutrophil recruitment depend on the injury, any reduced liver injury will cause less neutrophil accumulation in the liver, but this does not prove that neutrophils actually caused damage. In contrast, various studies on neutrophil function during APAP hepatotoxicity have shown no relevant activation of hepatic or circulating neutrophils and no protection when neutrophils are inactivated [8,13]. More importantly, neutrophil activation in APAP overdose patients occurs not during the injury phase, when alanine aminotransferase values are rising, but only during the regeneration phase, when alanine aminotransferase and liver injury parameters are falling, and resolution of the injury and regeneration begins [13]. As priming and activation, especially for reactive oxygen formation, is required for neutrophil cytotoxicity [4], these data suggest the primary role of neutrophils during APAP hepatotoxicity occurs after the injury phase [13]. Similar data were also reported for infiltrating monocytes in animals [14] and patients [15].

As such, it is unlikely that sterile inflammation plays a major role in the pathophysiology of APAP overdose. It is substantially more likely that inflammation during APAP-induced liver injury serves as a pro-regenerative measure that clears necrotic debris and paves the way for liver cell regeneration [8].

Cholestatic liver injury

Cholestatic liver injury occurs during a number of different liver pathologies including obstructive cholestasis, primary biliary cirrhosis, primary sclerosing cholangitis and more [16]. Cholestasis results in retention of bile species in hepatocytes, which may elicit a number of compensatory changes in the liver. Since murine bile acids do not cause hepatocellular injury, neutrophil recruitment is caused by release of chemotactic factors such as biliary osteopontin and bile acid-induced CXC chemokine formation in hepatocytes [17]. Thus, the sterile inflammatory response is initiated independent of cell necrosis. However, neutrophils are responsible for most of the cell injury [18]. Although DAMPs such as HMGB1 are being released [19], TLR4 deficiency has no effect on the injury [17]. In contrast to the murine model, pathophysiologically relevant concentrations of human bile acids cause cell necrosis in human hepatocytes and release of DAMPs in patients with obstructive cholestasis directly [20]. These findings suggest that a sterile inflammatory response occurs in both mice and humans during cholestasis, albeit the initiating mechanisms are different. In addition, in contrast to the well-established role of neutrophils in the mouse bile duct ligation model [18], the importance of sterile inflammation in patients remains to be investigated.

Conclusions

While a number of studies have reported a role for sterile inflammation in the pathogenesis of liver injury, there remains a debate in the field as to how relevant some of these studies

actually are and how useful sterile inflammation may be as a therapeutic target. More work is required in this area to resolve these issues, especially in regards to how much of the current information in animal models can accurately be translated to human patients.

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