REVIEW

The impact of sterile inflammation in acute liver injury

Benjamin L. Woolbright, Hartmut Jaeschke

Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, Kansas, United States

ARTICLE INFO

Article history:
Received: December 18, 2016
Revised: February 10, 2017
Accepted: February 12, 2017
Published online: February 12, 2017

Keywords:
sterile inflammation
liver
neutrophil
monocyte
mechanisms
cholestasis
ischemia
reperfusion
acetaminophen

ABSTRACT

Background: The liver has a number of functions in innate immunity. These functions predispose the liver to innate immune-mediated liver injury when inflammation goes unchecked. Significant progress has been made in the last 25 years on sterile inflammatory liver injury in a number of models; however, a great deal of controversy and many questions about the nature of sterile inflammation still exist.

Aim: The goal of this article is to review sterile inflammatory liver injury using both a basic approach to what constitutes the inflammatory injury, and through examination of current models of liver injury and inflammation. This information will be tied to human patient conditions when appropriate.

Relevance for patients: Inflammation is one of the most critical factors for managing in-patient liver disease in a number of scenarios. More information is needed for both scientists and clinicians to develop rational treatments.

1. Introduction

Liver associated morbidity and mortality is a major concern globally. The liver has a number of important functions in the body. One of the major functions of the liver is related to innate immunity, as the liver holds the largest macrophage population in the body, termed Kupffer cells (KCs). Diseases that result in acute liver injury, activate the liver’s innate immune functions, sometimes pathologically, even in the absence of an infection. Activation of KCs in the liver results in release of reactive oxygen species (ROS), and secretion of an array of cytokines that recruit other potentially cytotoxic inflammatory cells, including neutrophils, monocytes, T-cells, and NK/NKT cells [1]. The idea of innate immune-mediated liver injury in the absence of an infection has become a highly studied topic in liver biology over the last 25 years. While in some models there is nearly universal agreement that inflammation is a direct, pathological component of the injury process, there are other models wherein there is considerable debate over the role of sterile inflammation [2-5]. In a number of diseases, it is becoming increasingly understood that inflammation may actually play a beneficial, or at least a necessary role, as both acute and chronic liver failure can dramatically increase susceptibility to infection. The purpose of this review is to discuss mechanisms of sterile inflammatory liver injury and further discuss the role of sterile inflammation in clinically relevant models of acute liver injury.
2. Sterile inflammation – mechanisms of inflammation and injury

2.1 Release of damage associated molecular patterns

Sterile inflammation is a common outcome of a number of different clinical liver disorders. Sterile inflammation occurs in solid organs, such as the liver, when an organized inflammatory response occurs in the absence of any infection. While infectious inflammatory responses are driven by the detection and engulfment of infectious species, or pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide and flagellin, sterile inflammatory responses are driven by local release of damage associated molecular patterns (DAMPs) from dying, necrotic cells [1, 6]. As cells die, their plasma membrane becomes permeable to intracellular contents [7]. In the case of outright necrosis, rupture of the cell occurs, and intracellular contents, or DAMPs, spill into the surrounding area. In the classic model of sterile inflammation these molecules then bind to pattern recognition receptors such as Toll-like receptors (TLRs), which induce pro-inflammatory cytokine formation [3,6,8]. Other DAMPs can either prime, or activate the multimeric protein complex called the inflammasome [9,10]. A number of damage associated molecular patterns have been identified including: ATP [11], high mobility group box-1 (HMGB1) [12], mitochondrial DNA and nuclear DNA [13,14], uric acid [15], hyaluronan [16], histones [17, 18] and more. Additionally, some molecules, such as bile acids (BA), have a number of similar qualities to known DAMPs, and are released during cell death [19]. Moreover, as necrosis results in total cellular breakdown, any number of cellular components could also be functioning as DAMPs. Thus, the sterile inflammatory response during liver injury is initiated by breakdown of the hepatocyte membrane and release of these constituents. The presence and the detection of these molecules drives the inflammatory response.

2.2 Activation of Kupffer cells

KCs are an endogenous macrophage population present in the sinusoidal space of the liver vasculature. Release of DAMPs activates a number of potential receptors on KCs. KCs express TLRs including TLR3, TLR4, TLR9 and more [20]. Additionally, KCs express purinergic receptors [21], the hyaluronan receptor CD44 [22], the receptor for advanced glycation end products (RAGE), which acts as a receptor for HMGB1 [23], and potentially other unknown receptors that also mediate these effects. Binding of these receptors serves to activate KCs resulting in local production of a number of cytokines and chemokines, in addition to the NF-κB pathway which causes upregulation of a multiple pro-inflammatory proteins [20]. Release of TNF-α from KCs can also activate NF-κB in hepatocytes, which upregulates adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) in models of liver injury [24]. In addition, in mice, many of these cytokines and chemokines as such macrophage inflammatory protein-2 (MIP-2), mouse keratinocyte factor (mKC), and macrophage chemoattractant protein-1 (MCP-1) have their own receptors i.e. CXC chemokine receptor 1 (CXCR1), CXC chemokine receptor 2 (CXCR2), and C-C chemokine receptor 2 (CCR2), respectively, all of which have been implicated in specific liver diseases [25-30]. Parallel activation of a number of these pathways likely occurs and amplifies the signals. All of these agents serve to increase recruitment of other inflammatory cells such as neutrophils and monocytes in addition to stimulating other resident macrophages.

Activation of TLR signaling on KCs also initiates the two-step process responsible for activating the inflammasome [31]. The inflammasome is a multimeric complex of proteins that initiates the activation of caspase-1 from pro-caspase 1, and subsequent cleavage of pro-interleukin-1β (pro-IL-1β) to IL-1β, which serves as a potent pro-inflammatory cytokine [9]. While a number of different proteins can initiate formation of an inflammasome-like complex, the classical and most studied example of the inflammasome in the liver is the Nalp3 inflammasome [3]. Activation of TLR4, as an example, increases expression of pro-IL-1β through the NF-κB pathway [8]. When KCs encounter DAMP signals, such as ATP, which activates the purinergic receptor 2X7 (P2X7), a ligand-gated ion channel, it causes release of intracellular potassium and activation of caspase-1 by the NLRP3 inflammasome. The activated caspase-1 cleaves pro-IL-1β to form IL-1β and then IL-1β is released into the blood [9]. The NLRP3 inflammasome has been implicated in a number of models of liver injury, and will be discussed further in the context of specific diseases.

2.3 Initiation of inflammatory cell death – cytotoxic mediators of inflammation

Increased blood levels of cytokines and chemokines released from KCs result in rapid recruitment of multiple innate immune cells to the liver. The discussion will be mainly focused on the two cell populations that accumulate most commonly and in the highest numbers: neutrophils and monocytes/monocyte-derived macrophages. A brief section will highlight potential roles of other inflammatory cells as well. The liver recruits high numbers of both cell populations rapidly in response to injury. In models such as cholestatic liver injury [32,33], acetaminophen- (APAP) induced liver injury [34], hepatic ischemia-reperfusion liver injury [35], binge alcohol administration to chronically alcohol fed mice [36], and endotoxemia [37] there is rapid recruitment of neutrophils to the liver within 3-9 hours post initiation of toxicity. Monocytes typically follow shortly afterwards in some of these models [28]; however, it should be noted that KCs can also initiate toxicity and are endogenously present. Specific toxicity mechanisms of these two populations will be discussed in detail.

2.3.1 Kupffer cells and monocyte-derived macrophages

KCs are endogenously present in the liver and are a primary innate immune cell type responsible for considerable bacterial,
fungal, and viral clearance [38,39]. However, over-activation of KCs can be detrimental to hepatocyte function. KCs produce ROS through the enzyme NADPH oxidase (NOX2). Upon stimulation, KCs induce a vascular oxidant stress as evidenced by major increases in extracellular glutathione disulfide [40]. This is directly linked to hepatocyte cell death through activation of complement [41]. In support of these data, inactivation or removal of KCs with a number of different methods including administration of methyl palmitate, gadolinium chloride, or clodronate liposomes are protective against injury [40,42-46]. Given its established role in numerous diseases, release of ROS remains the most likely mechanism of toxicity from KCs.

In addition to production of ROS, KCs are frequently noted as potent producer of tumor necrosis factor-α (TNF-α), despite the fact that in a majority of liver diseases the actual increase in serum TNF-α levels is very limited. While there are models that are unequivocally mediated by release of TNF from KCs [47,48], in a majority of models, the release of TNF-α alone is insufficient to induce hepatocyte apoptosis. This is due to the fact that hepatocytes are highly resistant to TNF-α, and only undergo TNF-α mediated cell death in the presence of inhibition of nuclear factor kappa light chain enhancer of B cells (NF-κB) [49]. Human hepatocytes and human hepatocyte-like cell lines may be even more resistant to TNF-α, as they require even higher concentrations of the cytokine to induce apoptosis [50,51]. As such, TNF-α is likely less than oxidative stress to be relevant as a cytotoxic mediator of KC.

Monocyte-derived macrophages (MoMF) are a well-studied cellular population in the liver. During liver injury, outside monocytes get recruited to the liver, wherein they take on a macrophage-like phenotype with similar functions. Increasingly, it is being recognized that these monocytes fit one of two very different roles. Classically activated M1 or pro-inflammatory macrophages generally secrete pro-inflammatory cytokines, are Ly6C<sup>hi</sup>, exacerbate inflammation, and in some cases contribute to cell death [52]. Alternately activated M2 or anti-inflammatory type macrophages secrete large amounts of anti-inflammatory and pro-resolving cytokines like interleukin-10 (IL-10), are stimulated by IL-4, and serve to remove necrotic debris and resolve inflammation in preparation for regeneration [52,53]. Accumulation of M1 type macrophages in the liver would indicate monocytes could potentially cause inflammatory liver injury; whereas accumulation of M2 macrophages would suggest wound healing is beginning to occur and the injury phase is declining [54]. MoMF express the same subset of enzymes necessary for production of oxidative stress as KCs, and are hypothesized to produce toxicity in the same fashion.

Finally, it should be noted that despite its acknowledged roles in multiple disease states, modulation of the innate immune system, especially endogenous KCs, is risky as a therapeutic target. The primary purpose for KCs and other innate immune cells remains the clearance of bacteria. In patients with alcoholic hepatitis, blockade of TNF-α signaling resulted in increased risk of sepsis, and caused excess mortality [55,56]. Similarly, despite the fact that acute liver failure patients suffer from dramatically reduced liver function, a large percentage of patients do not die of hepatic insufficiency, but rather from sepsis associated with liver failure. Given this information, blockade of inflammation remains a controversial target at best in liver disease, and should only be pursued in patients without an active infection, or evidence of systemic inflammatory response syndrome (SIRS). Moreover, more specific therapeutic targets are needed that could potentially reduce the adverse effects of major immune activation, without inhibiting host defense. In spite of this, understanding sterile inflammation in different models is imperative to understanding disease progression and mortality in patients.

### 2.3.2 Neutrophils

Neutrophil-mediated solid organ injury has been studied extensively in liver and other organs, and requires multiple steps [2,57]. Neutrophil recruitment is necessary, and in high numbers neutrophils can generate sufficient oxidative stress to kill hepatocytes [58]. This is carried out by production of cytokines and chemokines in both mice and humans. Next, neutrophils have to adhere to the vasculature and extravasate into the parenchyma [59,60]. In most organs, neutrophils roll along post-capillary venules through interactions between selectins on endothelial cells and their ligands expressed on neutrophils [61]. Expression of these molecules is regulated by cytokines, and thus cytokines serve not only to recruit neutrophils, but to promote their attachment to the endothelium for transendothelial migration. However, in the liver, the only limited neutrophil rolling is present in post-sinusoidal venules [62,63]. In contrast, most neutrophil extravasation occurs from the sinusoids [64]. Both E-selectin [36,65], and their ligands [66], have been suggested to be involved in neutrophil mediated liver injury in different models, indicating E-selectin may still be involved in neutrophil transendothelial migration in sinusoids. Alternatively, the hepatoprotection by blocking selectins may be a secondary effect caused by reduced injury in the intestine in certain models such as ischemia-reperfusion injury [67]. Furthermore, it was hypothesized that P-selectin-dependent platelet accumulation during hepatic ischemia-reperfusion may cause microvascular perfusion failure and facilitate neutrophil accumulation in sinusoids [68,69]. However, the pathophysiological role of platelet-neutrophil interactions has recently been questioned [70].

Independent of the controversial role of selectins in the liver, neutrophil transmigration involves mainly interactions of LFA-1 (CD11a/CD18) or Mac-1 (CD11b/CD18) on neutrophils and ICAM-1 on sinusoidal endothelial cells [71]. Once neutrophils transmigrate past the endothelium, interactions between β2 integrins (LFA-1/Mac-1) and ICAM-1 on the target cells mediate neutrophil adhesion to target cells [72,73] including hepatocytes [74]. Firm adhesion to hepatocytes is required for toxicity as this triggers the long-lasting oxidant stress and the diffusion of ROS into hepatocytes [2,57]. Fur-
thermore, local anti-proteases nullify the toxic potential of neutrophil-derived proteases without very close physical proximity [75]. Neutrophil cytotoxicity requires upregulation of CD11b on neutrophils, and expression of the Mac-1 complex on the cellular surface of neutrophils [72], in addition to ICAM-1 upregulation on hepatocytes [76,77]. Blockade or deletion of either adhesion component results in a significant decrease in neutrophil toxicity in the liver [32,35,41] as does deletion of ICAM-1 [76-78]. As such, CD11b expression at the cellular surface is a common indicator of neutrophil activation [79]. Finally, when firmly adhered, neutrophils are capable of killing hepatocytes through degranulation and release of a number of cytotoxic species [80,81]. Neutrophils produce ROS through the NADPH oxidase system, which catalyzes the formation of superoxide by transferring electrons from NADPH to molecular oxygen and initiates toxicity [81]. Superoxide dismutates spontaneously or catalyzed by superoxide dismutase (SOD) into molecular oxygen and hydrogen peroxide, which can be toxic to hepatocytes [82]. In addition, neutrophils are the sole producer in mammalian biology of the enzyme myeloperoxidase.

Myeloperoxidase is present in neutrophil granules in the interior of the cell. Upon neutrophil activation, this enzyme is released into the local space where it reacts with chloride anion and hydrogen peroxide produced from NADPH oxidase to generate hypochlorous acid, a potent oxidant [82]. Hypochlorous acid is highly toxic and rapidly addsucts tyrosine, which causes significant oxidative stress and protein dysfunction [83]. These adducts are readily identifiable with antibodies against chlorotyrosine, and are present in a number of models of neutrophil mediated liver injury [32,78,81,84]. Additionally, neutrophils express elastase in their azurophilic granules, which can result in cellular dysfunction in some disease, such as emphysema of the lungs [85]. Elastase is a serine-protease thought to be involved in breakdown of extracellular matrix for neutrophil movement. Neutrophil elastase has been suggested to potentially be involved in liver injury [86]; although, mechanisms of how elastase could damage hepatocytes have not been elucidated, nor has there been any update on how elastase escapes the effect of local anti-proteases in the liver [75].

Recently it was identified that neutrophils are capable of secreting a mixture of granule proteins and neutrophil DNA, that form a mesh-like fibrous network known as a neutrophil extracellular trap (NET) [87]. These traps have a clear role in pathogen removal, wherein pathogens are trapped within the fibrous network and are subsequently cleared [87,88]. While this process is unequivocally important in immune function, whether or not NETs can serve as a cause of tissue injury or tissue pathology is poorly characterized. NET formation during sterile inflammation has been noted in multiple models [89,90]. NET formation may have a role in ischemic liver injury, as NETs are formed in ischemic liver lobes and treatment with DNase I both reduced liver injury, and reduced NET formation [91]. This likely occurs through a HMGB1-TLR4 mediated pathway, indicating sterile inflammation can serve as an activator of NET formation independent of bacteria [91]. More research is required in this area, along with more specific ways of targeting NET formation, to fully understand how NETs themselves might drive pathogenesis and damage hepatocytes.

Given these well-established mechanisms of neutrophil cytotoxicity in the liver [57,92], whether or not neutrophils directly cause injury in a model can be assessed by interventions that target these mechanisms. If neutrophils were causing injury, the above steps would happen prior to the major development of injury, biomarkers of neutrophil-mediated liver injury such as chlorotyrosine staining, CD11b upregulation, and localized neutrophil recruitment would be prevalent, and knockout of the critical components such as CD11a, CD11b, CD18, ICAM-1, LFA, or other adhesion molecules would provide protection in relevant models.

### 2.3.3 Other inflammatory liver cells

In addition to the populations of macrophages and recruited neutrophils, the liver also contains a large population of natural killer (NK) cells of various types including: NK cells, NKT cells, and γδT cells [93]. Natural killer cells produce a number of cytokines that can exacerbate inflammation, but primarily are known for secreting interferon-gamma (IFN-γ) and TNF-α [93]. NK cells have cytotoxic activity as their granules contain a number of cytotoxic proteases that can directly perforate cells causing cell death, and they can also present pro-apoptotic proteins such as Fas ligand and CD40L [94]. NK cells have been implicated as potential players in the inflammatory environment of multiple types of acute liver injury including cholestatic liver diseases [95], APAP-induced liver injury [96], and hepatic ischemia-reperfusion injury [97], although the most common explanation is not as an effector cell, but rather as a stimulant for increased pro-inflammatory cytokine production. The role of NK cells in APAP induced liver injury has been particularly controversial with groups attaining opposite results. Jc18 mice that are deficient in Vα14iNKT cells are also resistant to APAP induced liver injury but this was attributed to changes in metabolism of APAP by the Jc18 mice that preferentially detoxified APAP [98]. These results were also challenged though as studies with CD1d knockout and Jc18 mice later indicated these mice underwent more severe injury due to stabilization and subsequent increases in CYP2E1 levels and thus greater biotransformation of APAP to its reactive metabolite [99]. As such, there is considerable debate in the field as to what the role of NK/NKT/iNKT cells truly is. Additional research is ongoing in this area as to whether or not subpopulations of these cells can potentially modulate the inflammatory environment during acute liver injury.

Dendritic cells have also been implicated in sterile inflammation in the liver. Dendritic cells are antigen presenting cells associated with the adaptive immune system that bridge the gap between innate immunity and adaptive immunity by presenting antigen material to local T cells [100]. The role of dendritic cells in acute sterile inflammation has not been well
explored. There is some evidence that depletion of dendritic cells exacerbates APAP-induced liver injury although the mechanism through which this occurs is not confirmed [101].

3. Sterile inflammation – models

It is difficult to exactly duplicate a clinical liver disease in mice. Despite this, a number of models have been developed that approximate the clinical liver disease present in patients. Many of these, such as APAP-induced liver injury and hepatic ischemia-reperfusion injury are high fidelity models that have informed clinical practice and dramatically sped up the rate of identifying potential therapeutic targets. These models remain imperative to our understanding of the associated human conditions and, as such, defining mechanisms of injury and inflammation in these models accurately is necessary for clinical advancement. Herein, we will discuss sterile inflammation in the context of multiple diseases in an attempt to understand how inflammation affects liver injury.

4. Hepatic ischemia-reperfusion injury

Liver transplantation and liver resection are becoming increasingly common interventions for patients with advanced liver disease or liver tumors, respectively. Both transplantation and resection require clamping the hepatic portal triad, and thus induce ischemia, followed by unclamping and reperfusion of the liver. This procedure can cause cell injury during ischemia (due to lack of oxygen and absence of blood flow) and during reperfusion (due to the reintroduction of oxygen and blood flow). The overall pathophysiology of hepatic ischemia-reperfusion injury is complex [39,102-104]. Hepatic ischemia-reperfusion injury is a classical and widely accepted example of sterile inflammation and inflammatory liver injury [57,102,103] (Figure 1). The basic mechanisms that control liver injury in this model serve as a guide.

4.1 Ischemic liver injury

Clamping of the vasculature leads to immediate changes in hepatocytes. Primarily, the lack of oxygen shifts metabolism away from oxidative phosphorylation and towards glycolysis. This results in depletion of ATP, and a dramatic increase in intracellular constituents such as lactate, increased intracellular osmolarity, and reduced pH due to accumulation of H+ cations [100,105]. Eventually, these cells will undergo necrosis due to accumulation of calcium, and breakdown of membrane potential, however, there is no formation of reactive oxygen species [107,108]. Under realistic clinical conditions during elective surgery, the ischemic time will be limited in order to avoid severe ischemic injury. Nevertheless, hepatic ischemia together with early events during reperfusion, which include cell swelling caused by hyperosmolarity in the cytosol due to the accumulated metabolites, will lead to cellular stress and release of intracellular content [109]. Once the accumulated metabolites are either metabolized or flushed out, the swelling will subside. If there was no mitochondrial damage during ischemia, there will be limited intracellular oxidant stress during reperfusion [108]. On the other hand, if there was ischemic damage, the intracellular post-ischemic oxidant stress will be derived from xanthine oxidase, and more importantly, from damaged mitochondria [110]. In patients, prolonged hepatic ischemia can trigger ischemic hepatitis, which has a very high mortality rate [111]. Biomarkers indicate that ischemic hepatitis involves severe necrosis with extensive mitochondrial damage [112].

4.2 Reperfusion injury – initiation

A majority of cell death occurs after reperfusion [113]. Hepatocytes are generally resistant to ischemia for long periods, in part due to the fact that the mitochondrial membrane permeability transition pore (MPTP) opening can be reversible [114]. However, once re-oxygenation begins, hepatocyte leakage and mild necrosis releases a number of DAMPs such as HMGB1 [115], and mitochondrial DNA [14]. HMGB1 release occurs as early as one hour post-reperfusion, and continues to increase for up to twelve hours later [116]. HMGB1 activates TLR4 on KCs [115] and hepatocytes [117]. In addition, complement is activated during this period which directly activates KCs to generate reactive oxygen and complement components are potent neutrophils activators [41]. Thus, inhibition of complement activation or depletion of complement factors is highly

Figure 1. A simple model of ischemia-reperfusion injury. In the ischemia phase (top), occlusion of the blood flow causes hepatocyte swelling and release of intracellular contents that activate local Kupffer cells that express TLR4. This causes release of ROS from KCs. In the reperfusion phase, localized production of chemokines and cytokines by KCs recruit and activate neutrophils. These neutrophils exacerbate the ROS produced by KCs. KC – Kupffer cell, PMN – neutrophil, HMGB1 – high mobility group box-1, ROS – reactive oxygen species, DAMPs – damage associated molecular patterns, ATP – adenosine triphosphate.

Distributed under creative commons license 4.0
DOI: http://dx.doi.org/10.18053/jctres.03.2017S1.003
protective against the injury [41]. KCs then produce ROS that can damage hepatocytes [41,118]. In addition, cytokines such as TNF-α and chemokines promote neutrophil recruitment [119,120]. In murine models, this also includes activation of the Nalp3 inflammasome through an apoptosis-associated speck-like protein (ASC), which exacerbates further inflammation [121]. Inflammasome activation may occur through TLR9 in this model, although a better characterization of inflammasome activating factors such as histones are needed [122]. A number of experimental interventions support the idea that KCs are critical for the initial injury and the progression of the inflammatory response [102]. The predominant oxidative stress occurs largely in the vasculature and not in parenchymal cells during reperfusion, indicating that hepatocytes do not contribute directly to the oxidative stress [40]. Inactivation of KCs dramatically reduces injury and vascular oxidant stress [40]. Preconditioning of the liver by pulsing short bursts of ischemia and reperfusion induces a number of anti-oxidant genes that are protective [123]. Administration of glutathione is also protective through the scavenging of the vascular oxidant stress [124-126]. A number of different pharmacological agents have been shown to act through either reduction of KC-mediated oxidative stress or through a direct action on KC activation [39,127]. After the initial oxidative stress from KCs, secreted cytokines, chemokines and activated complement factors further recruit neutrophils which produce more cytokines formation and more oxidant stress, leading to a vicious cycle of continued inflammation and injury [41,118-120,128,129].

4.3 Reperfusion injury – a second stage

Neutrophils exacerbate ischemia-reperfusion injury in the liver [35]. Neutrophil accumulation in the post-ischemic liver lobes is obvious and dramatic as early as three hours post-reperfusion [41]. Of note, neutrophils begin to upregulate CD11b as early as one hour post-reperfusion, consistent with a neutrophil mediated liver injury [130]. Inactivation of neutrophils with a Mac-1 monoclonal antibody is protective against the injury, even when administered only 30 minutes before the onset of ischemia [130]. Treatment of rats with an antibody against ICAM-1 is also protective [77], even in steatotic livers that are more prone to cell death after transplantation [126]. Protection against hepatic ischemia reperfusion with a number of agents is correlated strongly with a reduction in neutrophil recruitment or function [39]. Generally, inhibition of upstream mediators of inflammation such as HGBM1 [115], or mitochondrial DAMPs [14,131], prevents downstream neutrophil accumulation and is highly protective against injury. As such, neutrophils are the likely critical mediator of the second phase of injury. In addition, it has been noted that other inflammatory cells might be involved in the second phase. CD4+ T cells also accumulate rapidly in the liver [132]. The primary mechanism through which these cells injure hepatocytes and exacerbate injury is likely through enhanced recruitment of neutrophils in an IL-17 dependent fashion [133]. Interferon regulatory factor 3 (IRF3) might be the critical link between recruitment of T-cells and the initial oxidant stress, as IRF3−/− mice are strongly protected against hepatic ischemia reperfusion injury in an IL-17 dependent mechanism [134].

Cytokine expression profile during this period is integral to the injury as activation of CXCR4 [135], and CXCR2 [29] are both detrimental to liver health; whereas, a number of other interleukins and cytokines such as IL-33 [136], and IL-37 [137], as well as other KC-derived mediators such as secreted leukocyte protease inhibitor (SLPI) reduce and control excessive inflammation [138]. The inflammation is eventually resolved through these anti-inflammatory processes, largely via production of IL-10 and activation of NF-κB [139] as well as production of IL-4 and activation of signal activator and transducer 6 (STAT6) [140] and production of IL-6 [141]. Resolution of hepatic ischemia-reperfusion injury is critical to recovery from both resection and liver transplantation, which is apparent in clinical practice by the substantial number of anti-inflammatory and immuno-modulatory drugs given in both settings [142].

5. Acetaminophen-induced liver injury

By far the most controversial topic in the field of sterile inflammation is the role of inflammation in APAP-induced liver injury. Despite the fact that there has been an effective antidote for over 30 years to prevent APAP-induced liver injury in early presenting patients, there still is no antidote for preventing late-stage APAP hepatotoxicity, or APAP-induced acute liver failure (ALF). Administration of N-acetylcysteine (NAC) replenishes glutathione (GSH) levels, which can first scavenge the reactive metabolite N-acetyl p-benzoquinone imine (NAPQI) and later support ROS detoxification [143]. NAC is most effective at early time points. After APAP metabolism is complete, the efficacy of NAC is diminished [144]. To address this concern, many have proposed the idea that there is a second phase of injury associated with APAP overdose that is dependent on inflammatory cells, predominantly focused on KCs, neutrophils and monocyes [30,86,96,145-147]. Despite the obvious appeal and some experimental support, there is a considerable amount of evidence that also argues for the contrary [5,148].

5.1 Acetaminophen induced liver injury – intracellular oxidative stress and the initial phase of toxicity

The initial phase of toxicity has been extensively studied over the last 40+ years. Foundational work was performed by Mitchell and colleagues when they demonstrated that APAP overdose rapidly depletes GSH, causes APAP protein adducts and leads to hepatocellular necrosis [149]. Subsequent work has demonstrated a number of the intracellular signaling mechanisms responsible for both metabolism and the toxicity. APAP is rapidly glucurononidated by UDP-glucuronoxytransferase 1A1 and sulfated through sulfotransferase 2A1 [150]. During an overdose, only the sulfation but not glucuronidation pathway is saturated [151]. While neither of these processes

Distributed under creative commons license 4.0

DOI: http://dx.doi.org/10.18053/jctres.03.2017S1.003
produces a toxic metabolite, oxidation by cytochrome P4502E1 results in the formation of NAPQI [152], which can adduct proteins and trigger a profound mitochondrial oxidative stress [153]. The exact nature of which proteins become adducted remains a question in the field, but what is understood is that this intracellular oxidative stress mediates a substantial portion of the subsequent injury through a number of mechanisms. Foremost, the oxidative stress triggers activation of the c-Jun N-terminal kinase (JNK) pathway, and results in phosphorylation of JNK, and translocation of JNK to the mitochondria [154]. A number of other pathway members are also activated including Sab, MLK3, and Ask1, and also contribute to the injury process [155-157]. Knockout or pharmacological inhibition of these proteins is protective, as is knockout or pharmacological inhibition of JNK itself [158]. In addition to JNK activation, the oxidative stress activates the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway [159]. This results in upregulation of a number of cytoprotective molecules that mediate autoprotection upon further APAP dosing [159]. Knockout of Nrf2 dramatically exacerbates injury [159], and knockout of the Nrf2 binding factor Kelch like-ECH-associated protein 1 (Keap1) is highly protective against APAP-induced liver injury [160]. This same oxidative stress also activates a number of different B-cell lymphoma-2 (Bcl-2) family proteins including Bcl-2 associated X-protein (bax) and BH3 interacting death domain agonist (bid) [161,162]. Both of these factors translocate to the mitochondria where they form pores in the outer mitochondrial membrane and help to initiate the mitochondrial permeability transition (MPT). Simultaneous to these events, is activation of the receptor interacting kinase 1/receptor interacting kinase 3 (RIP1/RIP3) pathway [163-165]. In the classical necroptotic pathway, these proteins form a complex called the ripoptosome that can activate mixed lineage kinase domain-like (MLKL), which then translocates to the cell membrane and forms membrane-disrupting pores leading to necrotic cell death [166]. However, recent studies showed that MLKL<sup>−/−</sup> mice are not protected against APAP hepatotoxicity suggesting that APAP-induced cell death is not a necroptotic mechanism [165,167]. Although there seems to be no role for MLKL and thus direct necroptosis in APAP-induced liver injury, yet knockout of either RIP1 or RIP3 seems to be protective [163,165]. While the current role of these proteins is not well understood in APAP toxicity, it is believed that this complex might have other functions that promote cytotoxicity. Regardless, the primary event that occurs subsequent to all these events is MPT formation in the mitochondria [168]. JNK translocation to the mitochondria dramatically enhances the mitochondrial oxidative stress and stimulates mitochondrial dysfunction and pore opening [169,170]. This causes complex cellular dysfunction and reduces ATP stores for the cell [171]. Moreover, the mitochondrial oxidative stress results in leakage of mitochondrial membrane proteins such as apoptosis-inducing factor (AIF), and endonuclease G [172,173]. Both proteins are nucleases and translocate the nucleus where they directly attack DNA and eventually cause nuclear DNA lysis and cell death [173]. The primary oxidative stress and GSH depletion occurs within the first two hours, and the secondary oxidative stress occurs for the following 4-12 hours. During this secondary period, stimulation of autophagy also occurs, and autophagy can protect against APAP induced liver injury by removing damaged mitochondria that leak cytotoxic proteins [174,175]. Autophagy also removes protein adducts which reduces the initial harmful stimuli and further protects against future cell death [176]. This is especially apparent in cells distal from the central vein area where the primary site of toxicity is, as these cells express lower quantities of CYP2E1, generate fewer adducts, and undergo less oxidative cell death [177]. This initial phase of toxicity is supported by dozens of papers, interventions, and mouse models that all point towards the idea that protein adducts serve as a central source of oxidative stress that targets the mitochondria and eventually causes cellular necrosis due to DNA lysis and failed mitochondria.

5.2 Sterile inflammation – a second phase of injury after APAP overdose?

5.2.1 Neutrophils

The idea of a second phase of injury has been controversial in the literature. The primary hypothesis that has been put forth many times is that recruited inflammatory cells, predominantly monocytes and neutrophils, exacerbate the initial injury through the release of reactive oxygen species and other cytotoxic compounds consistent with sterile inflammatory liver injury. This occurs after the initial oxidative stress, typically reported between 6 and 12 h after the initial overdose of APAP. Studies in favor of this hypothesis have presented a number of points that indicate depletion of inflammatory cells, depletion of specific inflammatory mediators and chemotactic factors, or pharmacological inhibition of specific mediators of the injury is protective against acetaminophen-induced liver injury [30,86,96,142,178-182]. Moreover, during the inflammatory injury phase there is accumulation of a number of these factors including the release of DAMPs, chemokine and cytokine formation, substantial neutrophil and monocyte recruitment, and co-localization of these factors with areas of necrosis [30,86,96,142,178-182]. As such, at first glance, the combination of these studies strongly supports this idea and argues in favor of a sequential sterile inflammatory response that exacerbates APAP-induced liver injury.

Despite the considerable evidence presented in these studies, there are a number of unresolved issues with this hypothesis. Foremost, there is still a substantial controversy over the obtained results in this field, with many studies not being repeatable by others laboratories [5]. The first report of a potential neutrophil-mediated cytotoxicity after APAP overdose roundly rejected the hypothesis as there was no evidence in favor of upregulation of CD11b, shedding of L-selectin, or other evidence of neutrophil activation during the toxicity phase, nor was there protection when a CD18 antibody was administered to reduce recruitment, transmigration and cytotoxicity of neu-
This was challenged by a subsequent study that found that depletion of NK/NKT cells reduced liver injury [96], with the same authors later proposing that the neutrophil was the main mediator of the second phase of APAP-induced liver injury [96]. Both of these studies have contrary reports indicating that the results depend on a questionable experimental design. The protective effect of NK/NKT cell depletion appeared to depend on the presence of dimethyl sulfoxide (DMSO), i.e. in the absence of DMSO as solvent for APAP no effect of NK/NKT cells was detectable [183]. In addition, neutrophil depletion is only effective against APAP toxicity when the neutropenia-inducing antibody is given as a 24 hour pre-treatment [142,145,184], but not when the antibody is administered shortly after APAP [184]. Importantly, it takes only 30 minutes to an hour to completely deplete neutrophils using the Gr-1 antibody that was used in these studies [185]. Interestingly, pre-treatment with the neutrophil-depleting antibody causes a pre-conditioning effect which activates a number of cyto-protective genes [186]. Use of these neutrophil-depleting antibodies has been repeated in subsequent studies, with the authors coming to the same conclusions despite the obvious problems present in this system [182]. If the Gr-1 antibody was truly protective due to its neutropenia effect, then administration just before the initial toxicity would also be effective; however, the Gr-1 antibody is clearly not protective when administered shortly after APAP administration but before any evidence of liver injury [184]. It has been noted though, that neutrophil activation as measured by CD11b expression and priming for ROS occurs largely after the development of liver injury, both in mice and more importantly, in man [184,187,188]. Moreover, inhibition of NADPH oxidase activity by either chemical inhibitors or deficiency of the gp91phox subunit, which completely inhibits hypochlorous acid formation as it stops the production of superoxide, is not protective [184,188]. In addition to anti-CD18 antibody administration not being effective [34], gene deficiency of CD18 is also ineffective, once again showing that there is no neutrophil-mediated cytotoxicity [187]. Similarly, it was proposed that the deletion of the CXCR receptor 2, which binds neutrophil chemoattractant chemokines, was protected [189], although this was supposedly not through inhibition of neutrophils but expression of anti-apoptotic genes. However, apoptosis as mode of cell death during APAP hepatotoxicity has been roundly rejected on multiple occasions [190,191]. It has also been proposed that neutrophils mediate toxicity after APAP overdose through release of elastase [86], a serine protease whose primary purpose is to enhance transendothelial migration through the breakdown of extracellular matrix components such as elastin. Elastase is largely present in azurophilic granules present in neutrophils. The authors of this manuscript failed to assess neutrophil activation, a required component of degranulation, and only reported that mice with elastase-deficient bone marrow cells were protected with no explanation as to how the actual cytotoxicity might occur [86]. Furthermore, in our hands, elastase-deficient mice were not protected against APAP toxicity, nor was a pharmacological inhibitor of elastase protective (Jaeschke and Woolbright, unpublished). In addition to these direct interventions against neutrophils, other studies that affected the availability of various DAMPs such as uric acid, or complement, had a substantial effect on hepatic neutrophil accumulation but did not attenuate liver injury [192,193]. Given the totality of this data, it is clear that there has yet to be a study to conclusively demonstrate the role of neutrophils as a feed-forward amplification loop of APAP-induced liver injury. The current data surrounding the role of neutrophils is largely based on experimental approaches that have been questioned. At the present time, there is no conclusive evidence for a relevant role of neutrophil cytotoxicity in this model and most importantly in patients [188].

5.2.2 Macrophages and monocytes

Similarly, there are multiple reports on the efficacy of reducing either endogenous macrophage function or recruited monocyte function; however, these reports came to drastically different conclusions despite the fact the same strains of mice and same chemicals were used to derive these results. It was reported that pre-treatment with gadolinium chloride, a compound that inactivates KCs and attenuates ROS formation [96], prevented almost 100% of APAP toxicity [178]. However, these findings have never been confirmed by others [194-196]. Gadolinium chloride treatment may have additional off-target effects and its use has caused controversy in other models [43,197]. Moreover, using a separate approach to eliminate KC activity, clodronate liposomes, caused the opposite result, i.e., aggravated APAP hepatotoxicity [195]. In addition, mice deficient in the gp91phox gene, a component of the NADPH oxidase, showed the same oxidant stress and liver injury as wild type animals [188,198]. Taken together, these findings suggest that KCs are not directly involved in the injury.

Of note, monocytes and MoMF have also been investigated repeatedly with highly variable results [27,28,145,199-201]. Despite using the same mice, these groups obtained opposite results in regard to the role of the macrophage recruitment receptor CCR2 in APAP-induced liver injury with groups finding no differences in injury between CCR2-/- and wild type mice [27,28], groups finding exacerbation of injury in CCR2-/- mice compared to wild type animals [145,199] and one group finding protection against the injury in CCR2-/- mice [30]. Variable results such as this remain unexplained in the literature. Importantly, results obtained with MCP-1-/- mice matched the findings in the CCR2-/- mice in one of the studies, indicating that CCR2/MCP-1 signaling is likely not involved in the injury [27]. In support of the animal studies, the role of macrophages has been investigated in patients [202-204]. Macrophages display a distinct M2 phenotype in APAP overdose patients [204]. This applies to both endogenous macrophages, which are proliferative after injury, and bone marrow-derived monocytes that are recruited via CCL2/MCP-1 [204]. These
data correlate with data from the mouse, wherein it was shown that recruited macrophages are also of the M2 phenotype and more importantly, tissue regeneration and repair after APAP overdose was delayed in CCR2−/− mice [27,28,200]. Moreover, high levels of secretory leukocyte protease inhibitor released from hepatocytes and macrophages inhibit immune function and increase susceptibility to sepsis and infection in APAP overdose patients [204]. Given this anti-inflammatory, pro-regenerative phenotype, macrophages are likely a key mediator of recovery, and preventing their recruitment and inactivation would be ill-advised. Even still, more reproducible effects in preclinical models are needed before any reliable conclusions can be drawn, and pitfalls in the experimental systems need to be identified and avoided.

5.2.3 The inflammasome

Although the evidence in favor of any specific inflammatory cell being the mediator of APAP-induced liver injury is limited in comparison to the evidence against it, there is also considerable evidence both for and against the role of specific mediators of inflammation, including a number of chemokines and cytokines. One of the foremost ideas in the field is that the inflammasome is primed through DAMPs binding to toll like receptors, e.g. TLR4 and TLR9, and actually activated by other DAMPs such as ATP, and that this activation mediates the injury through production of IL-1β [3,28,29,181,182]. In support of this, there are obvious increases in serum mitochondrial DNA [13], and serum ATP [205], after APAP overdose, and there is a modest increase in pro-IL-1β expression and serum IL-1β levels after APAP overdose -179, 180, 206. In addition, the formation of IL-1β but not pro-IL-1β (IL-1β mRNA) is inhibited by a caspase inhibitor [206]. Furthermore, the supposed inhibition of TLR4 by benzyl alcohol can suppress IL-1β signaling and protect against the injury [180]. Finally, there is a modest increase in protection in a number of models including NLRP3−/−, ASC−/−, P2X7R−/−, and TLR9−/− mice in addition to protection by pharmacological inhibition of TLR9, or treatment with apyrase, or treatment with DNase I, to breakdown extracellular DNA and prevent TLR9 activation [21,179-182,205]. However, some of these reports also are controversial as attempts to repeat much of these data have failed. Foremost, protection against injury with NLRP3−/−, ASC−/−, caspase-1−/−, IL-1R−/− mice and treatment with caspase inhibitors failed to provide protection in subsequent studies [206,207]. Administration of IL-1β directly in high doses did not yield any increase in toxicity, despite an increase in neutrophil recruitment [206,207]. Treatment with P2X7R pharmacological antagonist yielded more protection than the corresponding gene knockout of P2X7R [21], and the inhibitor was subsequently shown to inhibit APAP metabolism, and thus its protective effects were independent of its alleged pharmacological effects on inflammasome activation [208]. Furthermore, evidence that extracellular ATP directly exacerbates APAP-induced cell death [205] has also been un-repeatable in both rodent and human hepatocytes [209]. Most importantly, human plasma samples from APAP overdose patients did not have any evidence of enhanced IL-1β formation at any point over the first seven days of injury [5]. Thus, the inflammasome is not a likely a therapeutic target for preventing APAP toxicity, and its role in human patients is limited at best.

5.2.4 Interleukins

In addition to IL-1β, APAP exposure causes upregulation of several additional pro- and anti-inflammatory members of the interleukin family. Knockout of the IL-10 gene exacerbated APAP-induced liver injury [210]; however, this increased injury was linked to induction of inducible nitric oxide synthase in the absence of IL-10 and not an immune cell-mediated injury, indicating the increased injury was due to an effect on intracellular signaling mechanisms as APAP overdose causes extensive peroxynitrite formation in the mitochondria [211,212]. Knockout of IL-6 did not affect the injury, but did increase the rate of hepatocyte regeneration [213]. This may be due to signaling interactions with macrophages as inhibition of monocyte recruitment slows the rate of regeneration [28]. The role of IL-4 has become controversial as groups have attained opposite results in different strains of mice [214,215]. IL-4 is protective in C57BL/6J mice as it sustains increases in glutathione synthesis and recovery that are responsible for detoxification of oxidant stress in APAP toxicity [214], and thus IL-4−/− mice develop more severe injury. IL-4 administration in this model reversed the deleterious effects [214]. Others have recently reported that in the Balb/C mouse IL-4 exacerbates injury by enhancing inflammation and glutathione depletion [215]. This was in direct contrast to the previous results, although no attempts were made to explain these opposite findings. Since the article by Ryan and coworkers used multiple approaches to assess the role of IL-4, it is likely that this cytokines is beneficial by promoting hepatic GSH recovery [214]. Nevertheless, it needs to be kept in mind that the effect of IL-4 may be strain-dependent.

6. Liver injury during obstructive cholestasis

Cholestasis is caused by a blockage in bile flow from the liver to the intestine. It occurs either within the internal bile ducts of the liver, or in the external bile duct that connects the liver to the intestine. Regardless of the location, substantial liver injury occurs when animals or human patients undergo severe obstructive cholestasis. Inflammation is a noted aspect of murine models such as bile duct ligation (BDL) in rats [216], or mice [32], the multi-drug resistance protein 2 (MDR2) knockout mouse [217], and in human cholestasis patients of various etiologies [51]. Previous studies have indicated that cholestatic liver injury is mediated by both bile acid accumulation [218], and through inflammation, with both KCs [43], and neutrophils [32] being critically involved. One of the remaining questions in the field is which of these processes stimulates the initial cell death, and which mechanisms and cell types are then responsible for cell death. Herein, we will discuss these.
mechanisms with a focus on the BDL model, due to its prevalence in the literature, while also focusing on human patients.

6.1 Cholestatic liver injury – bile acid induced toxicity

When hepatocytes are exposed to sufficient concentrations of specific bile acids, they can undergo a variety of changes including apoptosis [218], necrosis [51,219], dramatic upregulation of cytokines such as MIP-2 and mKC [25], and activation of signaling pathways through G-protein coupled bile acid receptor (TGR) and farnesoid X receptor (FXR) [220,221]. With the exception of the activation of the above signaling pathways, which seem to occur in both human and rodent cells, many of these other changes are largely dependent on the origin of hepatocytes and the associated, individual bile acid species. Hepatocytes derived from the rat rapidly undergo apoptosis upon exposure to high concentrations of glycochenodeoxycholic acid (GCDC), a bile acid that is predominantly found in humans [51,222]. While apoptosis is not considered as pro-inflammatory as necrotic cell death, due to the lack of release of DAMPs, it has been noted that apoptosis can cause inflammation through serving as a danger signal itself, and through activation of local phagocytic macrophages [43,52]. In contrast, a number of other mammalian cells, including humans, are highly resistant to GCDC-induced apoptosis and require high concentrations of bile acids to initiate cell death [51,219,223]. Instead, hepatocytes from both humans and mice undergo frank necrosis after exposure to high concentrations of bile acids and release DAMPs such as HMGB1 [51]. Moreover, the relative toxicity of individual bile acids is highly variable, and dependent on their side chains, and to which amino acid they are conjugated [224]. The overwhelming majority of bile acids in cholestatic mammals are conjugated to either taurine or glycine [225]; however, the degree to which mammals use each amino acid varies greatly [51,226]. This has a number of biological effects, as conjugation to taurine reduces toxicity, but seems to promote inflammation, at least in the mouse [25]. Many of the less toxic taurine-conjugated bile acids, such as taurocholic acid, accumulate significantly in both mice and humans [226], and strongly upregulate and promote a number of pro-inflammatory cytokines in the mouse [25]. Of note though, this interaction is less prominent in either primary human hepatocytes [51], or human HepaRG cells [227]. As such, accumulation of bile acids can affect sterile inflammation in a number of ways: 1) bile acid-induced apoptosis can stimulate inflammation by activating KCs through phagocytosis of apoptotic bodies [43]; 2) bile acid-induced necrosis can stimulate inflammation through DAMP release [51]; 3) bile acids can directly stimulate inflammation without causing cell death through an early growth response factor-1 (Egr-1)-dependent signaling pathway [25]; 4) accumulation of bile acids and biliary pressure can activate signaling molecules such as osteopontin that result in neutrophil recruitment [228]. All of these pathways and mechanisms are contextual, based both on which bile acids are administered or which bile acids accumulate in the model or human patient, and also what sort of rodent model or human patient is being examined.

6.2 Bile duct ligation – a consensus model of inflammatory liver injury

BDL results in an immediate increase in biliary pressure that initiates overload of the biliary tracts and causes rupture of the bile ducts and release of bile onto the hepatic parenchyma within 6 hours [229,230]. Neutrophil counts in the liver rise about this same time, and are largely localized to the area immediately surrounding the area of biliary infarction [33]. Concomitantly, neutrophil activity rises acutely after BDL, and neutrophils from BDL animals have elevated NADPH oxidase activity [216,231]. Previous work from our laboratory has demonstrated that deficiency of the adhesion molecules CD18 or ICAM-1 is 60–80% protective against BDL injury [32,78]. Presumably, this is due to the lack of observable neutrophil extravasation present in the knockout animals [32,78]. Mice deficient in the Fas receptor are also protected against BDL via a substantial reduction in immune activity [232]. Supporting these data, P-selectin glycoprotein-1+ mice with deficient neutrophil attachment are also protected against BDL [66]. Knockout of the pro-inflammatory factor osteopontin is highly protective at early time points suggesting that cleaved osteopontin may be the initial critical chemotactic factor for neutrophil accumulation after BDL [228]. Osteopontin is cleaved by matrix metalloproteinases, and inhibition of these enzymes is also protective [228,233,234]. Expansion of this inflammatory response over time appears to involve Th17 cells as administration of IL-17 worsens inflammation and injury [235], and knockout of cluster of differentiation 279 results in a dramatic decrease in IL-17 producing cells which correlated with protection from inflammation and injury [236]. Multiple models of mice with an attenuated inflammatory response are protected including the lpr mouse [232,237], and the Na+/H+ exchanger regulatory factor1+ mouse [238]. All of these studies point towards a single hypothesis – the inflammation associated with bile duct ligation mediates a significant portion of the injury [19,239].

Despite this, the driving agent for inflammation in the model is still poorly characterized [239] Inflammation after BDL largely occurs at, and around, the point of biliary infarction, that is, the point of rupture of the biliary ducts due to the increased mechanical pressure [33,240]. Given that these points of rupture are also the site of the overwhelming degree of the associated necrosis, it has been hypothesized that bile acids released from the bile ducts directly damage hepatocytes, resulting in DAMP release and inflammation [237]. Initially, the hypothesis was that bile acid accumulation in the BDL model resulted in apoptosis, which drove the inflammatory response and subsequent neutrophil infiltration which exacerbated the injury and the fibrosis [237]. This was subsequently questioned as there is a lack of apoptosis in the BDL model, and the injury proceeds predominantly through necrosis [33,240]. Apoptosis of normal
hepatocytes has only been convincingly shown in rat hepatocytes exposed to high concentrations of GCDC [51,222], however, GCDC is not typically found in rodent models [226]. Attempts at defining a bile acid-dependent cell death in the mouse showed conclusively that concentrations of bile acids that did accumulate in the mouse were largely non-toxic, as taurine conjugated bile acids did not elicit toxicity at concentrations of up to 10 mM, far higher than what was present in serum or bile of cholestatic mice [25,226]. Instead, exposure of primary murine hepatocytes to taurine-conjugated bile acids such as taurocholic acid (TCA) elicited a dramatic increase in CXC chemokine expression and upregulation of ICAM-1 [25], similar to what was observed in vivo after BDL [232,241]. In addition, deficiency of osteopontin resulted in nearly complete protection against necrosis at early time points during BDL, but the protection was not sustained at distal time points [226]. This suggests that cleaved osteopontin is an early chemotactic factor for neutrophils that is released into the parenchyma after BDL. Subsequently, the chemotaxis may be maintained by CXC chemokines generated by bile acid-exposed hepatocytes [239]. Exposure to hydrophobic bile acid does cause significant cellular stress as indicated by mitochondrial dysfunction [242,243]. This may result in the release of additional local factors responsible for pro-inflammatory mediator generation, as there is substantial HMGB1 release as early as 6 hours post BDL [33]. More studies are needed to better define the initiating factors why neutrophils target damaged or dying hepatocytes during cholestasis, especially in humans.

6.3 Obstructive cholestasis in human patients

Cholestasis in humans occurs in various pathophysiologicals. Acute obstructive cholestasis induced by gallstones is rarely a chronic concern as patients will commonly present to the hospital rapidly with abdominal pain. The obstruction can be removed by endoscopic retrograde cholangiopancreatography. However, patient populations exist that cannot safely undergo endoscopy, and a greater understanding of cholestasis in patients is required to generate therapeutic options. Acute obstructive cholestasis presents with dramatic increases in alkaline phosphatase, and bilirubin, in addition to increases in serum transaminases. Furthermore, very early studies understood that serum bile acid levels were also dramatically elevated in cholestatic patients [225]. The idea that elevated serum bile acids might induce toxicity was proposed and tested, whereupon it was noted that bile acids induce toxicity in human hepatocytes at concentrations between 500µM and 1mM GCDC, far higher than what is necessary in rats or mice [51]. Human hepatocytes are also highly resistant to taurine-conjugated bile acids, and more importantly, they do not upregulate ICAM-1 or chemokines upon exposure to TCA [51]. A number of subsequent studies have conclusively shown the concentration necessary for toxicity is multiple orders of magnitude above human serum values achieved during cholestasis [51,244,245]; however, biliary concentrations for GCDC and other glycine-conjugated bile acids are commonly in the mM range [51,246]. As such, it is possible that biliary concentrations of bile acids can directly elicit toxicity. As the characteristic “foamy necrosis” and biliary infarcts present in BDL are also present in human patients, biliary bile acid concentrations may be responsible for the initial toxicity in human patients and subsequent release of pro-inflammatory factors such as HMGB1 [51]. While a sterile inflammatory response during obstructive cholestasis is clearly present in laboratory animal and in human patients, there are obvious differences between the current rodent models and human patients. Further studies are also needed in human patients to more fully define if neutrophils, or other inflammatory mediators, exacerbate the disease.

7. Conclusions

A sterile inflammatory response is an important aspect of acute liver injury. As the liver is an innate immune organ by nature, there is a delicate balance between activation, over-activation and hyper-stimulation that is constantly ongoing. Future studies are needed to better define the mechanisms that drive a sterile inflammatory response in various models and the pathophysiological relevance for the progression of the injury and the critical involvement in the process of regeneration and recovery. In addition, the vital importance of inflammation as defense against infection needs to be taken into account. Only a more detailed understanding of these mechanisms in relevant animal models and patients will allow the identification of potential therapeutic targets that can be used to translate these findings in animals to patients without impacting normal host defense functions.

Disclosure

The authors declare no conflict of interest.

Acknowledgements

Work in our laboratory was supported by a CTSA grant from NCATS awarded to the University of Kansas Medical Center for Frontiers: The Heartland Institute for Clinical and Translational Research # UL1TR000001 (formerly #UL1RR033179). This work was also supported in part by the National Institutes of Health grants R01 DK102142, DK070195, AA12916, and by grants from the National Institute of General Medical Sciences (P20 GM103549 and P30 GM118247) of the National Institutes of Health. Additional support came from an award (to BLW) from the ‘Training Program in Environmental Toxicology’ T32 ES07079-26A2 from the National Institute of Environmental Health Sciences.

References

[1] Krenkel O, Mossanen JC, Tacke F. Immune mechanisms in acetaminophen-induced acute liver failure. Hepatobiliary Surg Nutr 2014; 3: 331-343.
[2] Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. J Gastroenterol Hepatol 2011;
Sterile inflammation in the liver. Gastroenterology 2012; 143: 1158-1172.

[5] Woolbright BL, Jaeschke H. Sterile inflammation in acute liver injury: myth or mystery? Expert Rev Gastroenterol Hepatol 2015; 9: 1027-1029.

[6] Woolbright BL, Jaeschke H. Role of the Inflammasome in Acetaminophen-induced Liver Injury and Acute Liver Failure. J Hepatol 2017 in press.

[7] Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 1995; 146; 3-15.

[8] Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 1999; 274: 10689-10692.

[9] Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro-IL-beta. Mol Cell 2002; 10: 417-426.

[10] Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, Newton K, Qu Y, Liu J, Heddens S, Zhang J, Lee WP, Roose-Girma M, Dixit VM. Non-canonical inflammasome activation targets caspase-11. Nature 2011; 479: 117-121.

[11] Andrei C, Dazzi C, Lotti L, Torrisi MR, Chimini G, Rubartelli A. The secretory route of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles. Mol Biol Cell 1999; 10: 1463-1475.

[12] Gardella S, Andrei C, Ferrera D, Lotti LV, Torrisi MR, Bianchi ME, Rubartelli A. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-medicated secretory pathway. EMBO Rep 2002; 3: 995-1001.

[13] McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest 2012; 122: 1574-1583.

[14] Zhang Q, Raoof M, Chen Y, Sunni Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010; 464: 104-107.

[15] Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006; 440: 237-241.

[16] Yamasaki K, Muto J, Taylor KR, Cogen AL, Audish D, Bertin J, Grant EP, Coyle AJ, Misaghi A, Hoffman HM, Gallo RL. NLRP3/cryopyrin is necessary for interleukin-1β (IL-1β) release in response to hyperalumina, an endogenous trigger of inflammation in response to injury. J Biol Chem 2009; 284: 12762-12771.

[17] Huang H, Evankovich J, Yan W, Nace G, Zhang L, Ross M, Liao X, Billiar T, Xu J, Esmon CT, Tsung A. Endogenous histones function as alarmins in sterile inflammatory liver injury through Toll-like receptor 9 in mice. Hepatology 2011; 54: 999-1008.

[18] Wen Z, Lei Z, Yao L, Jiang P, Gu T, Ren F, Liu Y, Gou C, Li X, Wen T. Circulating histones are major mediators of systemic inflammation and cellular injury in patients with acute liver failure. Cell Death Dis 2016; 7: e2391.

[19] Woolbright BL, Jaeschke H. Therapeutic targets for cholestatic liver injury. Expert Opin Ther Targets 2016; 20: 463-475.

[20] Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology 2008; 48: 322-335.

[21] Hogue R, Sohail MA, Salhanick S, Malik AF, Ghan A, Robson SC, Mehal WZ. P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol Gastrointest Liver Physiol 2012; 302: G1171-G1179.

[22] Kennel SJ, Lankford TK, Foote LJ, Shinpock SG, Stringer C. CD44 expression on murine tissues. J Cell Sci 1993; 104: 373-382.

[23] Smedsrød B, Melkko J, Araki N, Sano H, Horiuchi S. Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. Biochem J 1997; 322: 567-573.

[24] Jaeschke H. Cellular adhesion molecules: regulation and functional significance in the pathogenesis of liver diseases. Am J Physiol 1997; 273: G602-11.

[25] Allen K, Jaeschke H, Cupple BL. Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. Am J Pathol 2011; 178: 175-186.

[26] Clarke C, Kuboki S, Sakai N, Kasten KR, Tevar AD, Schuster R, Blanchard J, Caldwell CC, Edwards MJ, Lentsch AB. CXC chemokine receptor-1 is expressed by hepatocytes and regulates liver recovery after hepatic ischemia/reperfusion injury. Hepatology 2011; 53: 261-271.

[27] Dambach DM, Watson LM, Gray KR, Durham SK, Laskin DL. Role of CCR2 in macrophage migration into the liver during acetaminophen-induced hepatotoxicity in the mouse. Hepatology 2002; 35: 1093-1103.

[28] Holt MP, Cheng L, Ju C. Identification and characterization of infiltrating macrophages in acetaminophen-induced liver injury. J Leukoc Biol 2008; 84: 1410-1421.

[29] Kuboki S, Shin T, Huber N, Eismann G, Talloway E, Schuster R, Blanchard J, Edwards MJ, Lentsch AB. Hepatocyte signaling through CXC chemokine receptor-2 is detrimental to liver recovery after ischemia/reperfusion in mice. Hepatology 2008; 48: 1213-1223.

[30] Mossanen JC, Krenkel O, Ergen C, Govaere O, Liepelt A, Puengel T, Heymann F, Kalthoff S, Lefebvre E, Eulberg D, Luedde T, Marx G, Strassburg CP, Roskams T, Trautwein C, Tacke F. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatology 2016; 64: 1667-1682.

[31] Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol 2016; 16: 407-420.

[32] Gujral JS, Farhood A, Bajt ML, Jaeschke H. Neutrophils aggravate acute liver injury during obstructive cholestasis in bile duct-ligated mice. Hepatology 2003; 38: 355-363.

[33] Woolbright BL, Antoine DJ, Jenkins RE, Bajt ML, Park BK, Jaeschke H. Plasma biomarkers of liver injury and inflammation demonstrate a lack of apoptosis during obstructive cholestasis in mice. Toxicol Appl Pharmacol 2013; 273: 527-531.

[34] Lawson JA, Farhood A, Hopper RD, Bajt ML, Jaeschke H. The hepatic inflammatory response after acetaminophen overdose: role of neutrophils. Toxicol Sci 2000; 54: 509-516.

[35] Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J 1990; 4: 3355-3359.

[36] Bertola A, Park O, Gao B. Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury
in mice: a critical role for E-selectin. Hepatology 2013; 58: 1814-23.

[37] Jaeschke H, Farhood A, Smith CW. Neutrophil-induced liver cell injury in endotoxin shock is a CD11b/CD18-dependent mechanism. Am J Physiol 1991; 261: G1051-1056.

[38] Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int 2006; 26: 1175-1186.

[39] Jaeschke H, Woolbright BL. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. Transplant Rev (Orlando) 2012; 26: 103-114.

[40] Jaeschke H, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. Am J Physiol 1991; 260: G355-362.

[41] Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol 1993; 264: G801-G809.

[42] Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RG. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. Hepatology 1994; 20: 453-460.

[43] Canbay A, Feldstein AE, Higuchi H, Werneg R, Gambhirer A, Bronk SF, Gores GJ. Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. Hepatology 2003; 38: 1188-1198.

[44] Liu P, McGuire GM, Fisher MA, Farhood A, Smith CW, Jaeschke H. Activation of Kupffer cells and neutrophils for reactive oxygen formation is responsible for endotoxin-enhanced liver injury after hepatic ischemia. Shock 1995; 3: 56-62.

[45] Marzi I, Cowper K, Takes Y, Lindert K, Lemasters JJ, Thurman RG. Methyl palmitate prevents Kupffer cell activation and improves survival after orthotopic liver transplantation in the rat. Transpl Int 1991; 4: 215-220.

[46] Zhong Z, Connor H, Mason RP, Qu W, Stachlewitz RF, Gao W, Lemasters JJ, Thurman RG. Destruction of Kupffer cells increases survival and reduces graft injury after transplantation of fatty livers from ethanol-treated rats. Liver Transpl Surg 1996; 2: 383-387.

[47] Tieg S, Wolter M, Wendel A. Tumor necrosis factor is a terminal mediator in galactosamine/endotoxin-induced hepatitis in mice. Biochem Pharmacol 1989; 38: 627-631.

[48] Bautista AP, Skrepnik N, Niesman MR, Bagby GJ. Elimination of macrophages by liposome-encapsulated dichloromethylene diphenylphosphonate suppresses the endotoxin-induced priming of Kupffer cells. J Leukoc Biol 1994a; 55: 321-7.

[49] Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene 1999; 18: 6910-6924.

[50] McGill MR, Yan HM, Ramachandran A, Murray GJ, Rollins DE, Jaeschke H. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology 2011; 53: 974-982.

[51] Woolbright BL, Dorko K, Antoine DJ, Clarke JI, Gholami P, Li F, Kumer SC, Schmitt TM, Forster J, Fan F, Jenkins RE, Park BK, Hagenbuch B, Oyaee M, Jaeschke H. Bile acid-induced necrosis in primary human hepatocytes and in patients with obstructive cholestasis. Toxicol Appl Pharmacol 2015; 283: 168-177.

[52] Wang M, You Q, Lor K, Chen F, Gao B, Ju C. Chronic alcohol ingestion modulates hepatic macrophage populations and functions in mice. J Leukoc Biol 2014; 96: 657-665.

[53] Heymann F, Trautwein C, Tacke F. Monocytes and macrophages as cellular targets in liver fibrosis. Inflamm Allergy Drug Targets 2009; 8: 307-318.

[54] Ju C, Tacke F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. Cell Mol Immunol 2016; 13: 316-327.

[55] Naveau S, Chollet-Martin S, Dharunacy S, Mathurin P, Jouet P, Piquet MA, Davison T, Oberti F, Broyt P, Emilie D; Foie-Alcool group of the Association Française pour l’Etude du Foie. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. Hepatology 2004; 39: 1390-1397.

[56] Boetticher NC, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, Boardman L, Gores GJ, Harmsen WS, McClain CJ, Kamath PS, Shah VH. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. Gastroenterology 2008; 135: 1953-1960.

[57] Jaeschke H. Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. Am J Physiol Gastrointest Liver Physiol 2006; 290: G1083-G1088.

[58] Jaeschke H, Ho YS, Fisher MA, Lawson JA, Farhood A. Glutathione peroxidase-deficient mice are more susceptible to neutrophil-mediated hepatic parenchymal cell injury during endotoxemia: importance of an intracellular oxidant stress. Hepatology 1999; 29: 443-450.

[59] Jaeschke H, Farhood A, Fisher MA, Smith CW. Sequestration of neutrophils in the hepatic vasculature during endotoxemia is independent of beta 2 integrins and intercellular adhesion molecule-1. Shock 1996; 6: 351-356.

[60] Jaeschke H, Fisher MA, Lawson JA, Simmons CA, Farhood A, Jones DA. Activation of caspase 3 (CPP32)-like proteases is essential for TNF-alpha-induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model. J Immunol 1998; 160: 3480-3486.

[61] McEver RP. Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol 2002; 14: 581-586.

[62] Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, Kubas P. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest 1997; 99: 2782-2790.

[63] Eassani NA, Fisher MA, Simmons CA, Hoover JL, Farhood A, Jaeschke H. Increased P-selectin gene expression in the liver vasculature and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. Leukoc Biol 1998; 63: 288-296.

[64] Chosay JG, Eassani NA, Dunn CJ, Jaeschke H. Neutrophil margination and extravasation in sinusoids and venules of liver during endotoxin-induced injury. Am J Physiol 1997; 272: G1195-G1200.

[65] Lawson JA, Burns AR, Farhood A, Lynn Bajt M, Collins RG, Smith CW, Jaeschke H. Pathophysiologic importance of E- and L-selectin for neutrophil-induced liver injury during endotoxemia in mice. Hepatology 2000; 32: 990-998.

[66] Dold S, Laschke MW, Zaslau Y, Schilling M, Menger MD, Jeppsson B, Thorlacius H. P-selectin glycoprotein ligand-1-mediated leukocyte recruitment regulates hepatocellular damage in acute obstructive cholestasis in mice. Inflamm Res 2010; 59: 291-298.

[67] Kubes P, Payne D, Woodman RC. Molecular mechanisms of leukocyte recruitment in postschismic liver microcirculation. Am J Physiol Gastrointest Liver Physiol 2002; 283: G139-G147.

[68] Yadav SS, Howell DN, Steeber DA, Harland RC, Tedder TF, DOI: http://dx.doi.org/10.18053/jctres.03.2017S1.003
Clavien PA. P-Selectin mediates reperfusion injury through neutrophil and platelet sequestration in the warm ischemic mouse liver. Hepatology 1999; 29: 1494-1502.

Khandoga A, Biberthaler P, Messmer K, Krombach F. Platelet-endothelial cell interactions during hepatic ischemia-reperfusion in vivo: a systematic analysis. Microvasc Res 2003; 65: 71-77.

van Golen RF, Stevens KM, Colarutto P, Jaeschke H, Heger M. Platelet aggregation but not activation and degradation during the acute post-ischemic reperfusion phase in livers with no underlying disease. J Clin Transl Res 2015; 1: 107-115.

Jaeschke H, Smith CW. Mechanisms of neutrophil-induced parenchymal cell injury. J Leukoc Biol 1997; 61: 647-653.

Entman ML, Youker K, Shoji T, Kukielka G, Shappell SB, Taylor AA, Smith CW. Neutrophil induced oxidative injury of cardiac myocytes. A compartmented system requiring CD11b/CD18-ICAM-1 adherence. J Clin Invest 1992; 90: 1335-1345.

Diamond MS, Stautton DE, de Fougerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA. ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). J Cell Biol 1990; 111: 3129-3139.

Nagendra AR, Mickelson JK, Smith CW. CD18 integrin and CD54-dependent neutrophil adhesion to cytokine-stimulated human hepatocytes. Am J Physiol 1997; 272: G408-G416.

Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320: 365-376.

Xu H, Gonzalez JA, St Pierre Y, Williams IR, Kupper TS, Crotan RS, Springer TA, Gutierrez-Ramos JC. Leukocytosis and resistance to septic shock in intercellular adhesion molecule-1-deficient mice. J Exp Med 1994; 180: 95-109.

Farhood A, McGuire GM, Manning AM, Miyasaka M, Smith CW, Jaeschke H. Intercellular adhesion molecule 1 (ICAM-1) expression and its role in neutrophil-induced ischemia-reperfusion injury in rat liver. J Leukoc Biol 1995; 57: 368-374.

Gujral JS, Liu J, Farhood A, Hinson JA, Jaeschke H. Functional importance of ICAM-1 in the mechanism of neutrophil-induced liver injury in bile duct-ligated mice. Am J Physiol Gastrointest Liver Physiol 2004; 286: G499-G507.

Zimmerman GA1, McIntyre TM. Neutrophil adherence to human endothelium in vitro occurs by CD18 (Mo1, MAC-1/LFA-1/ICAM-1) 150, 95 glycoprotein-dependent and independent mechanisms. J Clin Invest 1988; 81: 531-537.

Lehnert M, Arteel GE, Smutney OM, Conzelmann LO, Zhong Z, Thurman RG, Lemasters JJ. Dependence of liver injury after hemorrhage/resuscitation in mice on NADPH oxidase-derived superoxide. Shock 2003; 19: 345-351.

Gujral JS, Hinson JA, Jaeschke H. Chlorotyrosine protein adducts are reliable biomarkers of neutrophil-induced cytotoxicity in vivo. Comp Hepatol 2004; 3 Suppl 1: S48.

Kato Y. Neutrophil myeloperoxidase and its substrates: formation of specific markers and reactive compounds during inflammation. J Clin Biochem Nutr 2016; 58: 99-104.

Hazell LJ, Arnold L, Flowers D, Waeg G, Malle E, Stocker R. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. J Clin Invest 1996; 97: 1535-44.

Gujral JS, Hinson JA, Farhood A, Jaeschke H. NADPH oxidase-derived oxidant stress is critical for neutrophil cytotoxicity during endotoxemia. Am J Physiol Gastrointest Liver Physiol 2004; 287: G243-G252.

Sandhaus RA, Turino G. Neutrophil elastase-mediated lung disease. COPD 2013; 10 Suppl: 1: 60-3.

Huebener P, Pradere JP, Hernandez C, Gwak GY, Caviglia JM, Ma X, Loike JD, Jenkins RE, Antoine DJ, Schwabe RF. The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis. J Clin Invest 2015; 125: 539-550.

Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science 2004; 303: 1532-1535.

Clark SR, Ma AC, Tavenier SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercroe E, Devinney R, Doig CJ, Green FH, Kubes P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med. 2007; 13: 463-469.

Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science 2015; 349: 316-320.

Ge L, Zhou X, Ji WJ, Lu RY, Zhang Z, Zhang YD, Ma YQ, Zhao JH, Li YM. Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: therapeutical potential of DNA-based reperfusion strategy. Am J Physiol Heart Circ Physiol 2015; 308: H500-H509.

Huang H, Tohme S, Al-Khafaji AB, Tai S, Loughran P, Chen L, Wang S, Kim J, Biliar T, Wang Y, Tsung A. Damage-associated molecular pattern-activated neutrophil extracellular trap exacerbates sterile inflammatory liver injury. Hepatology 2015; 66: 600-614.

Jaeschke H, Hasegawa T. Role of neutrophils in acute inflammatory liver injury. Liver Int 2006; 26: 912-9.

Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. Immunol Today 2000; 21: 573–583.

Swain MG. Hepatic NKT cells: friend or foe? Clin Sci (Lond) 2008; 114: 457-466.

Shimoda S, Harada K, Niito H, Shirabe K, Taketomi A, Maehara Y, Tsukeyama K, Nakamura Y, Leung P, Ansari AA, Gershwin ME, Akashi K. Interaction between Toll-like receptors and natural killer cells in the destruction of bile ducts in primary biliary cirrhosis. Hepatology 2011; 53: 1270-1281.

Liu ZX, Govindarajan S, Kaplowitz N. Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity. Gastroenterology 2004; 127: 1760-1774.

Feng M, Li G, Qian X, Fan Y, Huang X, Zhang F, Lu L. IL-17A-producing NK cells were implicated in liver injury induced by ischemia and reperfusion. Int Immunopharmacol 2012; 13: 135-140.

Downs I, Aw TY, Liu J, Adegboyega P, Ajuebor MN. Vu14iNKT cell deficiency prevents acetaminophen-induced acute liver failure by enhancing hepatic glutathione and altering APAP metabolism. Biochem Biophys Res Commun 2012; 428: 245-251.

Martin-Murphy BV, Kominsky DJ, Orlikcy DJ, Donohue TM Jr, Ju C. Increased susceptibility of natural killer T-cell-deficient mice to acetaminophen-induced liver injury. Hepatology 2013; 57: 1575-1584.

Plitas G, Burt BM, Stableford JA, Nguyen HM, Welles AP, DeMatteo RP. Dendritic cells are required for effective cross-presentation in the murine liver. Hepatology 2008; 47: 1343-1351.

Connolly MK, Ayo D, Malhotra A, Hackman M, Bedrosian AS,
Ibrahim J, Cieza-Rubio NE, Nguyen AH, Henning JR, Dorvil-Castro M, Pachtner HL, Miller G. Dermritic cell depletion exacerbates acetonamphen hepatotoxicity. Hepatology 2011; 54: 959-968.

Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol 2003; 284: G15-26.

van Golen RF, Reiners MJ, Olihof PB, van Gulik TM, Heger M. Sterile inflammation in hepatic ischemia/reperfusion injury: present concepts and potential therapeutics. J Gastroenterol Hepatol 2013; 28: 394-400.

Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weginski JW. Ischemia-reperfusion injury in liver transplantation—from bench to bedside. Nat Rev Gastroenterol Hepatol 2013; 10: 79-89.

Herman B, Gores GJ, Nienlansen AL, Kawamishi T, Harman A, Lemasters JJ. Calcium and pH in anoxic and toxic injury. Crit Rev Toxicol 1990; 21: 127-48.

Kehrer JP, Jones DP, Lemasters JJ, Farber JL, Jaeschke H. Mechanisms of hypoxic cell injury. Summary of the symposium presented at the 1990 annual meeting of the Society of Toxicology. Toxicol Appl Pharmacol 1990; 106: 165-78.

Amund I, King J, Owen DA, Schneider H, Lemasters JJ, Thurman RG. Fructose prevents hypoxic cell death in liver. Am J Physiol 1987; 253: G390-G396.

Jaeschke H, Smith CV, Mitchell JR. Hypoxic damage generates reactive oxygen species in isolated perfused rat liver. Biochem Biophys Res Commun 1988; 150: 568-574.

Jaeschke H, Smith CV, Mitchell JR. Reactive oxygen species during ischemia-reflow injury in isolated perfused rat liver. J Clin Invest 1988; 81: 1240-1246.

Jaeschke H, Mitchell JR. Mitochondria and xanthine oxidase both generate reactive oxygen species in isolated perfused rat liver after hypoxic injury. Biochem Biophys Res Commun 1989; 160: 140-147.

Horvatsch T, Trauner M, Fuhrmann V. Hypoxic liver injury and cholestasis in critically ill patients. Curr Opin Crit Care 2013; 19: 128-132.

Weemhoff JL, Woolbright BL, Jenkins RE, McGill MR, Sharpe MR, Olson JC, Antoine DJ, Curry SC, Jaeschke H. Plasma biomarkers to study mechanisms of liver injury in patients with hypoxic hepatitis. Liver Int 2017; 37: 377-384.

Jaeschke H, Lemasters JJ. Apoptosis versus necrotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology 2003; 125: 1246-1257.

Hu J, Ramshesh VK, McGill MR, Jaeschke H, Lemasters J. Low Dose Acetaminophen Induces Reversible Mitochondrial Dysfunction Associated with Transient c-Jun N-Terminal Kinase Activation in Mouse Liver. Toxicol Sci 2016; 150: 204-215.

Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DB, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005; 201: 1135-1143.

Yang M, Antoine DJ, Weemhoff JL, Jenkins RE, Farhood A, Park BK, Jaeschke H. Biomarkers distinguish apoptotic and necrotic cell death during hepatic ischemia/reperfusion injury in mice. Liver Transpl 2014a; 20: 1372-1382.

Nace GW, Huang H, Klune JR, Eid RE, Rosborough BR, Korff S, Li S, Shapiro RA, Stolz DB, Sodhi CP, Hackam DJ, Geller DB, Billiar TR, Tsung A. Cellular-specific role of toll-like receptor 4 in hepatic ischemia-reperfusion injury in mice. Hepatology 2013; 58: 374-387.

Jaeschke H, Bautista AP, Spolarics Z, Spitzer JJ. Superoxide generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia. Free Radic Res Commun 1991; 15: 277-284.

Colletti LM, Remick DG, Burch GD, Kunel SL, Strieter RM, Campbell DA Jr. Role of tumor necrosis factor-alpha in the pathophysiological alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 1990; 85: 1936-1943.

Lentsch AB, Yoshidome H, Cheadle WG, Miller FN, Edwards MJ. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and KC. Hepatology 1998; 27: 1172-1177.

Kamo N, Ke B, Ghaffari AA, Shen XD, Busuttil RW, Cheng G, Kupiec-Weglinski JW. ASC/caspase-1/IL-1β signaling triggers inflammatory responses by promoting HMGB1 induction in liver ischemia/reperfusion injury. Hepatology 2013; 58: 351-362.

Huang H, Chen HW, Evankovich J, Yan W, Rosborough BR, Nace GW, Ding Q, Loughran P, Beer-Stolz D, Billiar TR, Esmon CT, Tsung A. Histones activate the NLRP3 inflammasome in Kupffer cells during sterile inflammatory liver injury. J Immunol 2013; 191: 2665-2679.

Kukan M, Vajdova K, Korycky J, Nagyová A, Mehendele HM, Tmocv T. Effects of blockade of Kupffer cells by gadolinium chloride on hepatobiliary function in cold ischemia-reperfusion injury of rat liver. Hepatology 1997; 26: 1250-1257.

Bižer M, Paumgartner G, Gerbes AL. Glutathione protects the rat liver against reperfusion injury after hypothermic preservation. Gastroenterology 1999; 117: 200-210.

Liu P, Fisher MA, Farhood A, Smith CW, Jaeschke H. Beneficial effects of extracellular glutathione against endotoxin-induced liver injury during ischemia and reperfusion. Circ Shock 1994; 43: 64-70.

Nakano H, Nagasaki H, Barama A, Boutjema K, Jaeck D, Kumada K, Tatsuno M, Baek Y, Kitamura N, Suzuki T, Yamaguchi M. The effects of N-acetylcysteine and anti-intercellular adhesion molecule-1 monoclonal antibody against ischemia-reperfusion injury of the rat steatotic liver produced by a choline-methionine-deficient diet. Hepatology 1997; 26: 670-678.

Chu MJ, Vater H, Riecke AJ, Phillips AR, Bartlett AS. Impact of ischaemic preconditioning on experimental steatotic livers following hepatic ischaemia-reperfusion injury: a systematic review. HPB (Oxford) 2015; 17: 1-10.

Jaeschke H, Bautista AP, Spolarics Z, Spitzer JJ. Superoxide generation by neutrophils and Kupffer cells during in vivo reperfusion after hepatic ischemia in rats. J Leukoc Biol 1992; 52: 377-382.

Okaya T, Lentsch AB. Cytokine cascades and the hepatic inflammatory response to ischemia and reperfusion. J Invest Surg 2003; 16: 141-147.

Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ, Smith CW. Functional inactivation of neutrophils with a Mac-1 (CD11b/CD18) monoclonal antibody protects against ischemia-reperfusion injury in rat liver. Hepatology 1993; 17: 915-923.

Bamboat ZM, Balachandran VP, Ocuin LM, Obaid H, Plitas G, DeMatteo RP. Toll-like receptor 9 inhibition confers protection from liver ischemia-reperfusion injury. Hepatology 2010; 51: 621-632.
Oxidant stress; inflammation; fusion.

Laskin J., Eur J Immunol 2006.

Mukaida N. Opposite roles of neutrophils and macrophages in inflammation and innate immunity. Int Immunol 1985). N Engl J Med 1988.

of oral N-acetylcysteine in the treatment of acetaminophen hepatotoxicity in mice by Smilkstein MJ.

Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent. Hepatology 1997; 26: 1513-1520.

Schmidt SC, Hamann S, Langrehr JM, Höflich C, Mittler J, Jacob D, Neuhaus P. Preoperative high-dose steroid administration attenuates the surgical stress response following liver resection: results of a prospective randomized study. J Hepatobiliary Pancreat Surg 2007; 14: 484-492.

Saito C, Zwingmann C, Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. Hepatology 2010b; 51: 246-254.

Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med 1988; 319: 1557-1562.

Ishida Y, Kondo T, Kimura A, Tsuneyama K, Takayasu T, Mukaida N. Opposite roles of neutrophils and macrophages in the pathogenesis of acetaminophen-induced acute liver injury. Eur J Immunol 2006; 36: 1028-1038.

Laskin DL, Gardner CR, Price VF, Jollow DJ. Modulation of macrophage functioning abrogates the acute hepatotoxicity of acetaminophen. Hepatology 1995; 21: 1045-1050.

Liu ZX, Han D, Gunawan B, Kaplowitz N. Neutrophil depletion protects against murine acetaminophen hepatotoxicity. Hepatology 2006; 43: 1220-1230.

Jaeschke H, Williams CD, Ramachandran A, Bajit ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. Liver Int 2012; 32: 8-20.

Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 1973 Oct; 187: 211-217.

Hjelle JJ, Kaassen CD. Glucuronidation and biliary excretion of acetaminophen in rats. J Pharmacol Exp Ther 1984; 228: 407-413.

Xie Y, McGill MR, Cook SF, Sharpe MR, Winefield RD, Wilkins DG, Rolls DE, Jaeschke H. Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells. Xenobiotica 2015; 45: 921-929.

Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA 1984; 81: 1327-1331.

Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev 2012; 44: 88-106.

Hanawa N, Shinohara M, Saberi B, Gaarde WA, Han D, Kaplowitz N. Role of JNK translocation to mitochondria leading to inhibition of mitochondrial bioenergetics in acetaminophen-induced liver injury. J Biol Chem 2008; 283: 13565-13577.

Sharma M, Gadang V, Jaeschke A. Critical role for mixed-lineage kinase 3 in acetaminophen-induced hepatotoxicity. Mol Pharmacol 2012; 82: 1001-1007.

Xie Y, Ramachandran A, Breckenridge DG, Liles JT, Lebofsky M, Farhood A, Jaeschke H. Inhibitor of apoptosis signal-regulating kinase 1 protects against acetaminophen-induced liver injury. Toxicol Appl Pharmacol 2015; 286: 1-9.

Win S, Than TA, Min RW, Aghajan M, Kaplowitz N. c-Jun N-terminal kinase mediates mouse liver injury through a novel Sab (SH3BP5)-dependent pathway leading to inactivation of intramitochondrial Src. Hepatology 2016; 63: 1987-2003.

Du K, Xie Y, McGill MR, Jaeschke H. Pathophysiologic significance of c-jun N-terminal kinase in acetaminophen hepatotoxicity. Expert Opin Drug Metab Toxicol 2015; 11: 1769-1779.

Enamoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O’Connor T, Harada T, Yamamoto M. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci 2001; 59: 169-177.

Okawa H, Motohashi H, Kobayashi A, Aburatani H, Kessler TW, Yamamoto M. Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochem Biophys Res Commun 2006; 339: 79-88.

El-Hassan H, Anwar K, Macanas-Pirard P, Crabtree M, Chow SC, Johnson VL, Lee PC, Hinton RH, Price SC, Kass GE. Involvement of mitochondria in acetaminophen-induced...
Bajt ML, Farhood A, Lamasters JJ, Jaeschke H. Mitochondrial bax translocation accelerates DNA fragmentation and cell necrosis in a murine model of acetaminophen hepatotoxicity. J Pharmacol Exp Ther 2003; 302: 8-14.

Ramachandran A, McGill MR, Xie Y, Ni HM, Ding WX, Jaeschke H. Receptor interacting protein kinase 3 is a critical early mediator of acetaminophen-induced hepatocyte necrosis in mice. Hepatology 2013; 58: 2099-2108.

Zhang YF, He W, Zhang C, Liu XJ, Lu Y, Wang H, Zhang ZH, Chen X, Xu DX. Role of receptor interacting protein (RIP)1 on apoptosis-inducing factor-mediated necrosis during acetaminophen-evoked acute liver failure in mice. Toxicol Lett 2014; 225: 445-453.

Dara L, Johnson H, Suda J, Win S, Gaarde W, Han D, Kaplowitz N. Receptor interacting protein kinase 1 mediates murine acetaminophen toxicity independent of the necroosome and not through necroptosis. Hepatology 2015; 62: 1847-1857.

Zhang J, Yang Y, He W, Sun L. Necroosome core machinery: MLKL, Cell Mol Life Sci 2016; 73: 2153-2163.

Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Aman K, Vandenaheele P, Linkermann A, Poremba C, Schleicher U, Dewitz C, Krautwald S, Neurath MF, Becker C, Wirtz S. The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. J Clin Invest 2016; 126: 4346-4360.

Kon K, Kim JS, Jaeschke H, Lamasters JJ. Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology 2004; 40: 1170-1179.

Gunawan BK, Liu ZX, Han D, Hanawa N, Gaarde WA, Kaplowitz N. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. Gastroenterology 2006; 131: 165-178.

Saito C, Lamasters JJ, Jaeschke H. c-Jun N-terminal kinase modulates oxidant stress and peroxynitrite formation independent of inducible nitric oxide synthase in acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2010; 246: 8-17.

Williams CD, Koerner MR, Lampe JN, Farhood A, Jaeschke H. Mouse strain-dependent caspase activation during acetaminophen hepatotoxicity does not result in apoptosis or modulation of inflammation. Toxicol Appl Pharmacol 2011; 257: 449-458.

Bajt ML, Cover C, Lamasters JJ, Jaeschke H. Nuclear translocation of endonuclease G and apoptosis-inducing factor during acetaminophen-induced liver cell injury. Toxicol Sci 2006; 94: 217-225.

Bajt ML, Ramachandran A, Yan HM, Lebofsky M, Farhood A, Lamasters JJ, Jaeschke H. Apoptosis-inducing factor modulates mitochondrial oxidant stress in acetaminophen hepatotoxicity. Toxicol Sci 2011; 122: 598-605.

Ni HM, Bockus A, Boggess N, Jaeschke H, Ding WX. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatology 2012; 55: 222-232.

Williams JA, Ni HM, Haynes A, Manley S, Li Y, Jaeschke H, Ding WX. Chronic deletion and acute knockdown of parkin have differential responses to acetaminophen-induced mitochondrial and liver injury in mice. J Biol Chem 2015; 290: 10934-10946.

Ni HM, McGill MR, Chao X, Du K, Williams JA, Xie Y, Jaeschke H, Ding WX. Removal of acetaminophen protein adducts by autophagy protects against acetaminophen-induced liver injury in mice. J Hepatol 2016; 65: 354-362.

Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology 1999; 30: 186-195.

Imaeda AB, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Suterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. J Clin Invest 2009; 119: 305-314.

Cai C, Huang H, Whelan S, Liu L, Kautza B, Luciano J, Wang G, Chen G, Stratimirovic S, Tsung A, Billiar TR, Zuckerkraut BS. Benzyl alcohol attenuates acetaminophen-induced acute liver injury in a Toll-like receptor-4-dependent pattern in mice. Hepatology 2014; 60: 990-1002.

Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, Lopes GA, Russo RC, Avila TV, Melgaço JG, Oliveira AG, Pinto MA, Lima CX, De Paula AM, Cara DC, Leite MF, Teixeira MM, Menezes GB. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology 2012; 56: 1971-1982.

Marques PE, Oliveira AG, Pereira RV, David BA, Gomides LF, Saraiva AM, Pires DA, Novaes JT, Patrichio DO, Cisalpino D, Menezes-Garcia Z, Leezy WM, Chapman SE, Mahecha G, Marques RE, Guabiruba R, Martins VP, Souza DG, Mansur DS, Teixeira MM, Leite MF, Menezes GB. Hepatic DNA depo-sition drives drug-induced liver injury and inflammation in mice. Hepatology 2015; 61: 348-360.

Masson MJ, Carpenter LD, Graf ML, Pohl LR. Pathogenic role of natural killer T and natural killer cells inacetaminophen-induced liver injury in mice is dependent on the presence of dimethyl sulfoxide. Hepatology 2008; 48: 889-897.

Cover C, Liu J, Farhood A, Malle E, Waalkes MP, Bajt ML, Jaeschke H. Pathophysiological role of the acute inflammatory response during acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2006; 216: 98-107.

Kodal P, Wu P, Lahiji PA, Brown EJ, Maher JH. ANIT toxicity toward mouse hepatocytes in vivo is mediated primarily by neutrophils via CD18 Am J Physiol Gastroint Liver Physiol 2006; 291: G355-63.

Jaeschke H, Liu J. Neutrophil depletion protects against murine acetaminophen hepatotoxicity: another perspective. Hepatology 2007; 45: 1588-1589.

Williams CD, Bajt ML, Farhood A, Jaeschke H. Acetaminophen-induced hepatic neutrophil accumulation and inflammatory liver injury in CD18-deficient mice. Liver Int 2010; 30: 1280-1292.

Williams CD, Bajt ML, Sharpe MR, McGill MR, Farhood A, Jaeschke H. Neutrophil activation during acetaminophen hepatotoxicity and repair in mice and humans. Toxicol Appl Pharmacol 2014; 275: 122-133.

Hu B, Colletti LM. CXCR2 receptor-2 knockout genotype increases X-linked inhibitor of apoptosis protein and protects mice from acetaminophen hepatotoxicity. Hepatology 2010; 52: 691-702.

DOI: http://dx.doi.org/10.18053/jctres.03.2017S1.003
of multiple DAMPs and DAMP receptors. Toxicol Appl Pharmacol 2010; 34:127.[204]

Ito Y, Bethea NW, Ahril ER, McCuskey RS. Early hepatic microvascular injury in response to acetaminophen toxicity. Microcirculation 2003; 10: 391-400.

Knight TR, Jaeschke H. Peroxynitrite formation and sinusoidal endothelial cell injury during acetaminophen-induced hepatoxicity in mice. Comp Hepatol 2004 Jan 14; 3 Suppl 1: S46.

Hogaboam CM, Bone-Larson CL, Steinhauser ML, Matsukawa A, Gosling J, Boring L, Charo IF, Simpson KJ, Lukacs NW, Kunkel SL. Exaggerated hepatic injury due to acetaminophen challenge in mice lacking C-C chemokine receptor 2. Am J Pathol 2000; 156: 1245-1252.

Holt MP, Yin H, Ju C. Exacerbation of acetaminophen-induced disturbances of liver sinusoidal endothelial cells in the absence of Kupffer cells in mice. Toxicol Lett 2010; 194: 34-41.

You Q, Holt M, Yin H, Li G, Hu CJ, Ju C. Role of hepatic resident and infiltrating macrophages in liver repair after acute injury. Biochem Pharmacol 2013; 86: 836-843.

Antoniades CG, Berry PA, Davies ET, Hussain M, Bernal W, Vergani D, Wendon J. Reduced monocyte HLA-DR expression: a novel biomarker of disease severity and outcome in acetaminophen-induced acute liver failure. Hepatology 2006; 44: 34-43.

Antoniades CG, Khamri W, Abeles RD, Taams LS, Triantafyllou E, Possamai LA, Bernsmeier C, Mitry RR, O’Brien A, Gilroy D, Goldin R, Heneghan M, Heaton N, Jassem W, Bernal W, Vergani D, Ma Y, Quaglia A, Wendon J, Thursz M. Secretory leukocyte protease inhibitor: a pivotal mediator of anti-inflammatory responses in acetaminophen-induced acute liver failure. Hepatology 2014; 59: 1564-1576.

Antoniades CG, Quaglia A, Taams LS, Mitry RR, Hussain M, Abeles R, Possamai LA, Bruce M, McPhail M, Starling C, Wagner B, Barnardo A, Pomplun S, Auzinger G, Bernal W, Heaton N, Vergani D, Thursz MR, Wendon J. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. Hepatology 2012; 56: 735-746.
MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. Hepatology 2006; 44: 420-429.

[234] Kahraman A, Bronk SF, Cazanave S, Wernher NT, Mott JL, Contreras PC, Gores GJ. Matrix metalloproteinase inhibitor, CTS-1027, attenuates liver injury and fibrosis in the bile duct-ligated mouse. Hepatol Res 2009; 39: 805-813.

[235] O’Brien KM, Allen KM, Rockwell CE, Towery K, Luyendyk JP, Copple BL. IL-17A synergistically enhances bile acid-induced inflammation during obstructive cholestasis. Am J Pathol 2013; 183: 1498-1507.

[236] Li Me, Mennone A, Soroka C, Hagey L, Ouyang X, Weinman E, Beyer JL. Na(+)/H(+) exchanger regulatory factor 1 knockout mice have an attenuated hepatocellular inflammatory response and are protected from cholestatic liver injury. Hepatology 2015; 62: 1227-1236.

[237] Woolbright BL, Jaeschke H. Novel insight into mechanisms of cholestatic liver injury. World J Gastroenterol 2012; 18: 4985-4993.

[238] Gantt P, Trauner M, Fuchsbiel B, Zollner G, Wagner M, Marschall HU, Zatloukal K, Denk H. Oncosis represents the main type of cell death in mouse models of cholestasis. J Hepatol 2005; 42: 378-385.

[239] Kim ND, Moon JO, Slitt AL, Copple BL. Early growth response factor-1 is critical for cholestatic liver injury. Toxicol Sci 2006; 90: 586-595.

[240] Rolo AP, Palmeira CM, Holy JM, Wallace KB. Role of mitochondrial dysfunction in combined bile acid-induced cytotoxicity: the switch between apoptosis and necrosis. Toxicol Sci 2004; 79: 196-204.

[241] Sokol RJ, Dahl R, Devereaux MW, Yerushalmi B, Kobak GE, Gumprecht E. Human hepatic mitochondria generate reactive oxygen species and undergo the permeability transition in response to hydrophobic bile acids. J Pediatr Gastroenterol Nutr 2005; 41: 235-243.

[242] Trottier J, Bialek A, Caron P, Straka RJ, Heathcote J, Milkiewicz P, Barbier O. Metabolomic profiling of 17 bile acids in serum from patients with primary biliary cirrhosis and primary sclerosing cholangitis: a pilot study. Dig Liver Dis 2012; 44: 303-310.

[243] Trottier J, Bialek A, Caron P, Straka RJ, Milkiewicz P, Barbier O. Profiling circulating and urinary bile acids in patients with biliary obstruction before and after biliary stenting. PLoS One 2011; 6: e22094.

[244] Dilger K, Hohenester S, Winkler-Budenhofer U, Bastiaansen BA, Schaap FG, Rust C, Beuers U. Effect of ursodeoxycholic acid on bile acid profiles and intestinal detoxification machinery in primary biliary cirrhosis and health. J Hepatol 2012; 57: 133-140.