Decoding cell death signals in liver inflammation

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Summary

Inflammation can be either beneficial or detrimental to the liver, depending on multiple factors. Mild (i.e., limited in intensity and destined to resolve) inflammatory responses have indeed been shown to exert consistent hepatoprotective effects, contributing to tissue repair and promoting the re-establishment of homeostasis. Conversely, excessive (i.e., disproportionate in intensity and permanent) inflammation may induce a massive loss of hepatocytes and hence exacerbate the severity of various hepatic conditions, including ischemia-reperfusion injury, systemic metabolic alterations (e.g., obesity, diabetes, non-alcoholic fatty liver disorders), alcoholic hepatitis, intoxication by xenobiotics and infection, de facto being associated with irreversible liver damage, fibrosis, and carcinogenesis. Both liver-resident cells (e.g., Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells) and cells that are recruited in response to injury (e.g., monocytes, macrophages, dendritic cells, natural killer cells) emit pro-inflammatory signals including but not limited to - cytokines, chemokines, lipid messengers, and reactive oxygen species that contribute to the apoptotic or necrotic demise of hepatocytes. In turn, dying hepatocytes release damage-associated molecular patterns that, upon binding to evolutionary conserved pattern recognition receptors-activate cells of the innate immune system to further stimulate inflammatory responses, hence establishing a highly hepatotoxic feedforward cycle of inflammation and cell death. In this review, we discuss the cellular and molecular mechanisms that account for the most deleterious effect of hepatic inflammation at the cellular level, that is, the initiation of a massive cell death response among hepatocytes.

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Introduction

Hepatic inflammation, which accompanies the majority of acute and chronic liver disorders, is a complex process that originates in response to a variety of stress conditions [1]. As in most other organs, hepatic inflammation is put in place to protect hepatocytes from injury, to favor the repair of tissue damage, and to promote the re-establishment of homeostasis, de facto exerting consistent hepatoprotective effects. However, inflammatory responses that are too intense or fail to resolve (i.e., they become chronic) are near-to-invariably accompanied by a massive loss of hepatocytes and hence cause serious damage to the liver parenchyma (Supplementary Discussion) [2]. As myofibroblasts originating from hepatic stem cells take over and replace dead...
hepatocytes, unresolved inflammation can stimulate a fibrotic/cirrhotic response characterized by an irreversible decline in liver functions [3]. Alternatively, owing to the fact that hepatocytes are endowed with a consistent replicative potential and attempt at reconstituting the dead parenchyma (which explains the clinical success of the “split-liver” transplantation, in which two recipients receive one half of the organ from a single donor) [4], chronic inflammation significantly increases the risk for hepatic carcinogenesis [5]. Besides these two end-stage conditions, dysregulated inflammatory responses have been associated with most (if not all) hepatotoxic insults, including (i) ischemia/reperfusion (IR) injuries; (ii) alcohol overconsumption; (iii) intoxications by xenobiotics or heavy metals (e.g., Cu²⁺, Hg²⁺); (iv) bacterial, viral and parasitic infections; as well as (v) systemic alterations of mitochondria have been reported to accumulate in livers affected by IR injury, NASH, sepsis and Wilson’s disease [8–10]. This reflects, on the one hand the central role of hepatocytes in the systemic metabolism of carbohydrates and lipids as well as in the detoxification of xenobiotics, and on the other hand the critical functions of mitochondria at the hub of intracellular metabolism, Ca²⁺ homeostasis, and cell death regulation [11].

Here we discuss the major deleterious consequence of liver inflammation at the cellular level, that is the massive loss of functional hepatic parenchyma, focusing on the cellular and molecular players involved in the initiation of this phenomenon.

**Cell death signals in the liver**

Extrinsic and intrinsic apoptosis as well as regulated necrosis (Fig. 1 and Supplementary Discussion) have been implicated in the death of hepatocytes as triggered by a variety of hepatotoxic insults such as viral and bacterial infections [12], metabolic disorders [13], alcohol overconsumption [14], and intoxication by xenobiotics [15].

**Extracellular signals**

Members of the tumor necrosis factor (TNF) protein superfamily, including TNFα, CD95L (also known as FASL) and TNF-related apoptosis-inducing ligand (TRAIL, official name TNFSF10) are among the most prominent and best characterized inducers of hepatocyte death. TNFα is produced in massive amounts by hematopoietic cells exposed in vivo to living bacteria or lipopolysaccharide (LPS), de facto accounting for the fatal shock syndrome that develops in these conditions (which includes a prominent hepatic component) [16]. Of note, both the fulminant hepatotoxic effects and the systemic lethality of TNFα are consistently decreased in Ripk3−/− mice [17], suggesting that regulated necrosis may play an important role in this setting, which has long been considered as a pure apoptotic model.

TNFα is a highly pleiotropic cytokine, inducing biological effects as diverse as cell proliferation, metabolic activation, inflammatory responses and (apoptotic or necrotic) cell death [18]. Thus, ligand-bound TNR receptor 1 (TNFR1) can elicit distinct signal transduction cascades, depending on several factors. Generally, the stabilization of TNFR1 trimers by TNFα is rapidly connected to the production of reactive oxygen species (ROS) (via the plasma membrane NAPDH oxidase NOX1 as well as by mitochondrial and cytosolic sources) [19] and to the activation of NF-κB, orchestrating a pro-survival and pro-inflammatory response [20]. In this setting, the NF-κB-mediated transactivation of caspase-8 and FADD-like apoptosis regulator (CFLAR, best known as c-FLIP) blocks cell death signals at the level of TNFR1 [21], hence allowing for the secretion of several pro-inflammatory mediators including interleukin (IL)-6 and IL-8 [20]. Of note, the acute phase protein IL-6 has been recently suggested to play a prominent role in hepatic carcinogenesis as it activates signal transducer and activator of transcription 3 (STAT3) [22]. Upon internalization, the composition of the supramolecular complexes assembled around TNFR1 changes, generally allowing for
Fig. 1. Molecular mechanisms of apoptosis. Extrinsic apoptosis can lead to either ligation of death receptors (e.g., CD95) or drop in the concentration of dependence receptor (e.g., PTHCH1) ligands below a specific threshold. In particular, CD95 ligand (CD95L) stabilizes CD95 trimers, hence allowing for the assembly of a plasma membrane-associated supramolecular complex that favors the proximity-induced activation of caspase-8. In turn, active caspase-8 sets off the execution machinery of apoptosis by activating a proteolytic cascade involving caspase-3, -6, and -7. Conversely, PTHCH1 appears to mediate the activation of executioner caspases via caspase-8-dependent signal transduction cascade. Several intracellular stress conditions (e.g., DNA damage) are specifically sensed by small members of the BCL-2 protein family (BH3-only proteins), which activate mitochondrial outer membrane permeabilization (MOMP) by stimulating the pore-forming activity of BAX and BAK. Alternatively, MOMP can be initiated at the inner mitochondrial membrane by the unspecific opening of the “permeability transition pore complex” (PTPC). Both these lethal cascades can be held in check by anti-apoptotic BCL-2 proteins, which physically bind (hence inhibiting) not only their pro-apoptotic counterparts but also various PTPC components. MOMP is paralleled by the dissipation of the mitochondrial transmembrane potential (ΔΨm) and results in the liberation of several mitochondrial proteins such as cytochrome C (CYTC), which, together with the cytosolic adaptor APAF-1, generates the caspase-9-activating platform known as “apoptosome”; apoptosis-inducing factor (AIF), which exerts caspase-independent pro-apoptotic functions by mediating large-scale DNA fragmentation; and direct IAP-binding protein with inducer of necrosis (IAPs). Thus, MOMP activates both caspase-dependent and caspase-independent mechanisms of apoptosis. Of note, the caspase-8-mediated cleavage of the BH3-only protein BID constitutes a major link between the extrinsic and intrinsic apoptotic pathways. IBD, truncated BID.

Fig. 2. Tumor necrosis factor signaling. Similar to CD95 ligand, tumor necrosis factor α (TNFα) stabilizes pre-assembled TNFα receptor 1 (TNFR1) trimers, hence allowing for the assembly of a membrane-proximal supramolecular complex including (but not limited to) TRADD, FADD, receptor-interacting protein kinase 1 (RIPK1), as well as multiple cellular inhibitors of apoptosis proteins (cIAPs). In this setting, RIPK1 gets rapidly ubiquitylated (by cIAPs and other ubiquitin ligases) and hence triggers a signal transduction cascade that leads to the activation of NF-kB, in turn resulting in the production of anti-apoptotic and pro-inflammatory factors. Conversely, upon deubiquitylation (by cylindromatosis [CYLD] and other enzymes), RIPK1 can recruit either caspase-8, therefore setting off the apoptotic caspase cascade (see also Fig. 1) or, in caspase-incompetent settings, its homolog RIPK3, resulting in the assembly of the “necrosome”. In this latter case, both RIPK1 and RIPK3 become phosphorylated and activate a mixed lineage kinase domain-like (MLKL)-, phosphoglycerate mutase family member 5 (PGAM5)-, mitochondrial fragmentation-dependent signaling pathway leading to necrosis. CFLAR, caspase-8 and FADD-like apoptosis regulator; DRP1, dynamin-related protein 1; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; TAB, TAK1-binding protein; TAK1, transforming growth factor β (TGFβ)-associated kinase 1; Ub, ubiquitin.

The intraluminal accumulation of misfolded proteins significantly affects ER functions, including the regulation of Ca2+ homeostasis, and generally results in the activation of a multipronged adaptive mechanism known as ER stress and autophagy: The intraluminal accumulation of misfolded proteins significantly affects ER functions, including the regulation of Ca2+ homeostasis, and generally results in the activation of a multipronged adaptive mechanism known as...
unfolded-protein response (UPR) [32]. In mammalian cells, the UPR is elicited by three distinct sensors: endoplasmic reticulum to nucleus signaling 1 (ERN1, best known as inositol-requiring enzyme 1α [IRE1α]), activating transcription factor 6 (ATF6) and eukaryotic translation initiation factor 2α (eIF2α) kinase 3 (EIF2AK3, best known as PKR-related ER kinase [PERK]) [32]. Under physiological conditions, IRE1α, ATF6, and PERK engage in inhibitory interactions with the ER-resident chaperone glucose-regulated protein, 78 kDa (GRP78). Conversely, accumulating unfolded proteins competitively displace GRP78 from IRE1α, ATF6 and PERK, leading to their activation and eventually to the upregulation of mechanisms that attempt to re-establish homeostasis, mainly involving (i) a general (but not complete) translational arrest, (ii) the upregulation of factors that promote protein folding and degradation, and (iii) the activation of autophagy [32]. Autophagy is a homeostatic mechanism that is critical both in baseline conditions, for the maintenance of intracellular homeostasis [33], and during adaptive stress responses, when it exerts major cytoprotective functions [34].

When the ER stress is mild and/or temporary, the combined activation of the UPR and autophagy generally allows for the recovery of homeostasis [35]. Conversely, protracted and/or excessive signaling via ER stress sensors is associated with the initiation of a mitochondrial pathway of apoptosis that involves, among other proteins, the transcription factor C/EBP homologous protein (CHOP), the protein phosphatase 1 (PP1) activator growth arrest- and DNA damage-inducible gene 34 (GADD34), apoptosis signal-regulating kinase 1 (ASK1), and JNK [36]. Interestingly, at least part of the effects of JNK and ASK1 stem from their ability to phosphorylate anti-apoptotic members of the BCL-2 family [37], hence de-inhibiting Ca2+-modulatory (e.g., inositol 1,4,5-trisphosphate receptor) [38], pro-autophagic (e.g., Beclin 1) [39] and pro-apoptotic (e.g., BAX, BAK) factors [40]. Thus, JNK and ASK1 appear to regulate a complex crosstalk between mechanisms for the maintenance of intracellular homeostasis (such as ER stress and autophagy) and the adaptive organismal response for the removal of irredeemably compromised, and hence potentially dangerous cells (apoptosis).

A causal link between ER stress responses and the apoptotic demise of hepatocytes has been established in a wide panel of hepatic disorders, including viral infections [41], intoxication with bacterial toxins [42], IR injuries [43], transplantation [44], NASH and NAFLD [45]. Of note, the ER stress response is required for the perception of cell death as immunogenic [46], and (no less than) three distinct ER-resident chaperones, namely, calreticulin (CRT), ERP57 and GP96, have been shown to exert immunostimulatory effects [47,48]. It is therefore tempting to speculate, yet remains to be formally demonstrated, that ER stress inhibitors may be useful for preventing the unwarranted rejection of liver allografts.

ROS: ROS, including anion peroxide and hydrogen peroxide, are a normal side product of the mitochondrial respiratory chain (mainly produced cat complex I and II), and, as they constitute highly reactive and highly diffusible species, are involved in several signal transduction cascades and adaptive stress responses [11]. A quantitatively minor but functionally not less important fraction of intracellular ROS is produced by plasma membrane, cytosolic and reticular multicomponent NADPH oxidases (NOXs), including NOX1, which participates in TNFR1–dependent NF-κB activation, JNK activation, and necroptosis [49], by xanthine oxidase, which catalyzes one step of purine catabolism [50], and by enzymes of the cytochrome P450 family [51]. In the liver, the latter appear to account for a consistent proportion (if not for the majority) of intracellular ROS production, even in the absence of cytochrome P450 substrates [52].

In physiological settings, the cytotoxic potential of ROS is held in check by a diversified battery of mitochondrial, cytosolic, and peroxisomal antioxidant systems, including superoxide dismutases, catalases, peroxiredoxins, thioredoxins, glutathione and thiol-containing proteins [11]. In response to multiple stimuli, however, ROS levels exceed the buffer capacity of these systems, resulting first in the activation of an adaptive response aimed at the re-establishment of redox homeostasis coupled to an inflammatory response [53], and then in intracellular damage, progressive mitochondrial dysfunction (paralleling the oxidation of respiratory complexes and several other proteins involved in mitochondrial metabolism), and cell death [7]. In particular, intracellular ROS are known to affect the functions of key components of the permeability transition pore complex (PTPC) including voltage-dependent anion channel 1 (VDAC1) [54], adenine nucleotide translocases (ANTs) [55] and subunits of the F1F0 ATPase [56], hence stimulating PTPC opening, MOMP and cell death via intrinsic apoptosis. Of note, in some cell types including hepatocytes, ROS activate ASK1 and their cytotoxic potential is largely determined by its subcellular localization and interacting partners [57].

The implication of ROS in the death of hepatocytes exposed to toxic insults has been extensively documented, in vitro and in vivo. Thus, redox stress has been shown to constitute an etiological determinant of hepatotoxicity during IR [58,59], viral infection [60], endotoxemia [61] ethanol intoxication [62] and NASH [63], to mention a few examples. Conversely, ROS appear to protect cirrhotic hepatocytes from transforming growth factor β (TGFβ)-induced apoptosis [64], and to be (at least partially) responsible for the beneficial effects of (hepatic) ischemic preconditioning on hepatic and renal IR [65,66]. Finally, even in the absence of overt cell death, ROS-dependent alterations of mitochondrial metabolism in the liver have been associated with multiple metabolic conditions, including insulin resistance [67].

NO and RNS: Similar to ROS, nitric oxide (NO) and reactive nitrogen species (RNS) exert a large spectrum of biological effects including (but not limited to) the stimulation of cell growth and tissue regeneration, the control of vascular functions, and the induction of cell death [68]. The major intracellular source of NO is represented by NO synthases (NOS), which catalyze the NADPH- and O2-assisted conversion of L-arginine into citrulline and NO [69]. However, whereas the endothelial NOS (eNOS) is constitutively active and mainly mediates the beneficial effects of NO on vasculature, its inducible counterpart (iNOS) is upregulated in response to stress, catalyzing intense (and hence potentially dangerous) waves of NO production [69]. At the cellular level, excess NO and RNS resemble ROS in their capacity to impair energetic metabolism as they covalently modify a large panel of proteins including mitochondrial complex IV [70] and ANT [71] (both involved in mitochondrial respiration) as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (a glycolytic enzyme) [72], hence favoring the opening of the PTPC and cell death.

In line with these considerations, NO and RNS have been ascribed both hepatoprotective and hepatotoxic effects. For instance, NO has been suggested to participate in liver regeneration [73] and to protect hepatocytes from cell death at
The case of IR injury

IR is a biphasic process entailing a massive necrotic and apoptotic cell death response that is initiated at reperfusion, along with the most often unsuccessful attempt of cells to re-establish homeostasis via autophagy [77]. Hepatic IR is a particularly interesting model as its main pathological consequence, i.e., the massive loss of hepatocytes during reperfusion, originates from a complex crosstalk between ER stress, oxidative stress, and inflammation during ischemia. Hypoxia induces indeed a considerable overgeneration of ROS [78], in turn (i) directly stimulating the (at least partially) NF-κB-dependent transactivation of various pro-inflammatory mediators [53] and (ii) promoting protein unfolding and hence triggering the ER stress response (as documented by the activation of XBP1 and ATF6 in the parenchyma of livers undergoing IR) [44,45]. Interestingly, the hepatic ER stress response per se exerts pro-inflammatory functions, both as it allows for the activation of the pro-inflammatory hepatocyte-specific transcription factor CREBH [79] and as it sensitizes liver-resident macrophages to stimulation by Toll-like receptor 4 (TLR4) agonists as well as hepatocytes to cell death induction by TNFα [44]. In line with this notion, mice pretreated with 4-phenylbutyrate, a synthetic chaperone used as an ER stress inhibitor, were protected from hepatic IR while manifesting reduced pro-inflammatory responses [80]. Moreover, ischemic pre-conditioning has been associated with the upregulation of the IL-1 receptor antagonist IL-1RA, BCL-2 and NO [81], suggesting that the hepatoprotective effects of this maneuver stem from the inhibition of inflammation and cell death coupled to the activation of repair mechanisms. Of note, TLR4 agonists, which generally operate as adjuvants to promote immune responses, have been shown to reduce hepatic damage in steatotic livers maintained in UW preservation solution [82], implying that TLR4 signaling not always mediates hepatotoxic effects.

Taken together, these observations suggest that the hepatotoxic effects of IR originate from a complex crosstalk between hepatocytes, tissue-resident immune cells and immune cells that are recruited to the site of injury. In this setting, a major role is played by the ER stress response, by lipid signals and by damage-associated molecular patterns (DAMPs) that are released by dying hepatocytes, as discussed below.

Lipids, major pleiotropic signals of inflammation and cell death

Lipids constitute a structurally heterogeneous group of hydrophobic molecules that serve a wide variety of functions. Lipids are indeed prominent energy-storing molecules (leading to a much higher caloric yield, i.e., ~9 kcal/g, than carbohydrates and proteins, i.e., ~4 kcal/g), critically contribute to the formation of biological membranes, and participate in several signal transduction cascades [83]. The liver plays a central role in the systemic metabolism of lipids and several classes of lipids, including (but not limited to) prostaglandins, leukotrienes, ceramides and fatty acids, have been shown to mediate hepatotoxic effects while stimulating hepatic inflammation.

Prostaglandins

Cyclooxygenase 2 (COX2)-derived prostaglandins have been implicated in diverse physiopathological processes including cell proliferation and activation of inflammatory responses [84]. Most non-steroidal anti-inflammatory drugs (NSAIDs), including blockbusters such as acetylsalicylic acid, ibuprofen and sulindac, de facto operate as relatively unspecific COX inhibitors, blocking the conversion of arachidonic acid into prostaglandins [84]. As it stands, the implication of COX2 in hepatic diseases remains controversial. On the one hand, indeed, the hepatocyte-specific transgenic expression of COX2 has been shown to accelerate D-galactosamine/LPS-induced liver failure by a prostaglandin E (PGE) receptor 1, subtype EP1 (PTGER1)-dependent mechanism involving the activation of JNK2 [85], and PGE2 has been causally involved in NAFLD and NASH [86]. On the other hand, robust genetic data indicate that COX2 protects mice from fatal hepatitis as triggered by agonistic anti-FAS antibodies, presumably as it regulates the expression of the epidermal growth factor receptor (EGFR) [87]. Along similar lines, prostaglandins (and in particular PGE2) appear to mediate robust hepatoprotective functions against IR and acetaminophen hepatotoxicity [88,89].

Leukotrienes

Together with prostaglandins, leukotrienes, which are generated by the catalytic activity of the enzyme arachidonate 5-lipoxygenase (ALOX5), constitute the major products of arachidonic acid metabolism [90]. Besides participating in inflammatory responses, leukotrienes promote chemotaxis and regulate the contraction of tracheal smooth muscles, contributing to the pathogenesis of asthma [91]. The knockout of Alox5 reportedly ameliorates the steatotic, inflammatory, and fibrotic responses spontaneously developing in Apoe−/− mice [92] or resulting from the administration of CCl4 [93]. Along similar lines, chemical inhibitors of ALOX5 or 5-lipoxygenase-activating protein (FLAP, an ALOX5 interactor) have been shown to reduce CCl4-induced liver injury [94] and to limit the inflammatory infiltration of the hepatic parenchyma in several distinct models of NASH and NAFLD [92], perhaps as they induce the preferential demise of activated Kupffer cells. Similar to ALOX5, arachidonate 15-lipoxygenase, another enzyme involved in arachidonic acid metabolism, may also play a prominent role in the pathogenesis of NASH and NAFLD [95].

Ceramides

Ceramides are lipid messengers that accumulate in response to various stress conditions, either upon the hydrolysis of sphingomyelin by sphingomyelinases or via a 3-step biosynthetic pathway that mainly occurs at ER membranes [96]. Ceramides are well known for their capacity to control proliferation and apoptosis at a cell-autonomous level [97] and are now emerging as prominent regulators of systemic metabolism [96]. In particular, ceramides, which also accumulate in response to death receptor activation [98], promote direct mitochondriotoxic effects [99], stimulate ROS generation [100], and inhibit various anti-apoptotic signal transducers, including AKT [101]. Mice lacking acidic sphingomyelinase (aSMase) are significantly protected against the lethal hepatotoxic effects of D-galactosamine/LPS, TNFα and agonistic anti-FAS antibodies [102,103]; and imipramine, a chemi-
Review

Fatty acids and neutral fats

Fatty acids and neutral fats (including mono-, di-, and triglycerides) constitute prominent energetic substrates, central components of cell membranes and serve as precursors for the synthesis of several other molecules [83]. Nevertheless, the accumulation of fatty acids and neutral fats as a result of metabolic disorders (e.g., insulin resistance, which is generally accompanied by an increase in circulating fatty acids) or an unbalanced diet is toxic for several tissues, including the liver [107]. In these settings, as well as in response to various toxins and xenobiotics including ethanol, fatty acids and neutral fats (mainly di- and triglycerides) accumulate within hepatocytes, resulting in a peculiar alteration of the hepatic parenchyma known as steatosis [107]. Irrespective of its origin, steatosis is associated with (at least some extent) of hepatic damage, resulting from the pro-apoptotic activity of free fatty acids (FFAs) and (hepatocyte death-related) inflammation [108].

Results from recent studies involving leptin- (ob/ob) and leptin receptor-deficient (db/db) obese mice and multiple in vitro models of lipotoxicity [109,110] suggest that the lipotoxic effects of FFAs are mediated in part by mitochondrial apoptosis and in part by death receptor-transduced signals, but not by RIPK1-dependent signal pathways. In particular, FFA-induced apoptosis appears to involve the activation of the tumor suppressor protein p53 [111], the overexpression of TRAIL receptors [111], ROS overproduction [112], BAX-dependent lysosomal membrane permeabilization [113], as well as direct mitochondriotoxic effects that result from the local accumulation of modified lipids, such as oxidized cardiolipin [114] and ceramides [115]. In line with this notion, a combination of saturated fatty acids such as palmitic and oleic acid has been shown to sensitize hepatocytes to cell death induction by various stimuli [9]. In ob/ob mice, this manifests with morphological and structural alterations of hepatocyte mitochondria as well as with reduced levels of VDAC1 phosphorylation by glycogen synthase kinase 3β (GSK-3β), increasing the propensity of these organelles to accumulate Ca²⁺ ions and to undergo lethal MOMP [9]. In ob/ob mice, this manifests with morphological and structural alterations of hepatocyte mitochondria as well as with reduced levels of VDAC1 phosphorylation by glycogen synthase kinase 3β (GSK-3β), increasing the propensity of these organelles to accumulate Ca²⁺ ions and to undergo lethal MOMP [9].

The liver of HFD-fed mice is infiltrated by a consistent amount of immat
cule myeloid cells. Together with liver-resident Kupffer cells [117], these CD11b^Ly6ChighLy6G^ cells secrete TNFα cytokines such as IL-12 and interferon γ (IFNγ), hence facilitating the activation-induced death of local natural killer T (NKT) cells and initiating a vicious feedforward loop bridging inflammation and HFD-associated hepatotoxicity [118]. The existence of a physiopathologically relevant link between FFAs, apoptosis, and inflammation has been validated by an immunohistochemical study of hepatic biopsies from 84 NAFLD patients, demonstrating that the stage of disease correlates with increased p53 expression levels and decreased amounts of BCL-2 [119]. Taking this into account, it would be an oversimplification to consider all lipids as equally hepatotoxic. Indeed, while the lipotoxic potential of FFAs taken as a single class of lipids is established, (i) distinct FFAs differ in their ability to promote cell death, saturated and long lipids being generally viewed as more toxic than their non-saturated and short counterparts [119]; and (ii) neutral fats (in particular triglycerides) appear to be relatively inert, if not to activate an adaptive response that exerts hepatoprotective effects [109,120].

DAMPs, amplification signals for inflammation and cell death

DAMPs, also called alarmins, constitute a group of chemically heterogeneous molecules that are exposed/released by dying cells to operate as modulators (most often activators) of sterile inflammation [121] (Table 1). Of note, in physiological conditions, DAMPs serve “daily jobs”, i.e., they contribute to inflammation-unrelated cellular functions, in thus far resembling several components of the cell death machinery [122].

To date, molecules as diverse as nuclear and mitochondrial DNA [123–126], purine nucleotides (i.e., ATP, UTP) [127,128], uric acid [129,130], lipids (i.e., sphingosine-1-phosphate [S1P]) [131], nuclear factors (i.e., high-mobility group box 1 [HMGB1], IL-33) [124,132,133], mitochondrial N-formyl peptides [121,126], ER chaperones (i.e., CRT, Erp57, GP96) [47,48], heat-shock proteins (HSPs, e.g., HSPA1A, HSP90AA1) [48,134–136] cytoskeletal proteins (i.e., F-actin) [137] and S100 proteins [138] have been found to behave as DAMPs. In spite of such a structural and chemical heterogeneity, DAMPs share the property of being concealed from the microenvironment in physiological conditions and of being exposed and/or released in response to cellular damage [139]. Until recently, necrotic cells were considered as the most prominent, if not the only, source of DAMPs [139]. At least in part, this notion stemmed from the facts that the plasma membrane remains intact throughout apoptosis and that apoptotic corpses are normally cleared by phagocytes before secondary necrosis intervenes (and hence before their content can passively spill into the extracellular microenvironment), owing to the exposure of specific eat-me signals such as phosphatidylserine [140]. However, it is now clear that apoptosis can also be associated with the exposure/release of several DAMPs and hence can activate inflammatory or immune responses [46].

Interestingly, DAMPs may not always operate as pro-inflammatory mediators. For instance, IL-33, a cytokine that in healthy cells mainly functions as a transcription factor [133], appears to inhibit, rather than stimulate, inflammatory reactions elicited by dying cells, perhaps owing to its capacity of preferentially activating Th2 immune responses [141]. Moreover, the binding of generally pro-inflammatory DAMPs, such as HMGB1 and HSPs, to “alternative” receptors, such as CD24 or SIGLEC10, sialic acid-binding Ig-like lectin 10 (SIGLEC10), has been reported to mediate consistent anti-inflammatory effects [134].

The implication of specific DAMPs and cognate pattern recognition receptors (PRRs) in the pathogenesis of liver diseases has just begun to emerge. Thus, hepatocytes have been shown to secrete ATP, most likely via pannexin 1 channels, as they succumb to FFAs [142] and acetaminophen [143], promoting the P2Y2-dependent infiltration of neutrophils and hence aggravating hepatic damage [143]. The non-histone chromatin-binding
Table 1. Examples of DAMPs potentially involved in liver disease.

<table>
<thead>
<tr>
<th>DAMP</th>
<th>Function(s)</th>
<th>Receptor(s)</th>
<th>Emission pathway</th>
<th>Notes</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>ATP</td>
<td>Purine metabolites</td>
<td>NLRP3 inflammasome activators</td>
<td>P2RX7, P2Y2</td>
<td>Secreted via autophagy- and PANX1-dependent mechanisms</td>
<td>Involved in the pathogenesis of conA-induced acute liver injury</td>
</tr>
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<td>CRT</td>
<td>ER chaperones</td>
<td>CD91</td>
<td>Exposed upon ER stress via the Golgi secretory pathway</td>
<td>ER stress plays a causative role in several hepatic disorders</td>
<td>[46, 47]</td>
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<tr>
<td>F-actin</td>
<td>Cytoskeletal component</td>
<td>CLEC9a</td>
<td>Exposed upon PMP</td>
<td>Not yet formally involved in liver disease</td>
<td>[137]</td>
</tr>
<tr>
<td>HMGB1</td>
<td>Multifunctional nuclear factor</td>
<td>AGER, TLR4, CD24/S1P, SIGLEC10</td>
<td>Released upon NMP and PMP</td>
<td>Involved in the pathogenesis of acute liver failure, hepatic IR and HBV infection</td>
<td>[134, 145-149]</td>
</tr>
<tr>
<td>HSPs</td>
<td>Molecular chaperons</td>
<td>TLR2, TLR4</td>
<td>Released upon PMP or secreted via active, exosome-dependent routes</td>
<td>Implicated in the response to hepatic IR and other hepatotoxic conditions</td>
<td>[48, 134-136]</td>
</tr>
<tr>
<td>IL-33</td>
<td>Repressor of nuclear transcription</td>
<td>IL1RL1</td>
<td>Released upon NMP and PMP</td>
<td>Released during acute and chronic liver failure, hepatic IR and conA intoxication</td>
<td>[133, 158, 159]</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Gene expression</td>
<td>NLRP3, TLR9</td>
<td>Released upon mitochondrial damage and PMP</td>
<td>mtDNA can be detected in the circulation of patients undergoing acute liver failure</td>
<td>[125, 126, 151, 152]</td>
</tr>
<tr>
<td>nDNA</td>
<td>Gene expression</td>
<td>NLRP3, TLR9</td>
<td>Released upon NMP and PMP</td>
<td>Implicated in the fibrotic response to acetaminophen</td>
<td>[123, 124, 150]</td>
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<tr>
<td>N-formyl peptides</td>
<td>Mitochondrial poly-peptides Potent neutrophil activators</td>
<td>FPR1</td>
<td>Released upon mitochondrial damage and PMP</td>
<td>N-formyl peptides can be detected in the blood of patients undergoing acute liver failure</td>
<td>[121, 126, 152]</td>
</tr>
<tr>
<td>S100 proteins</td>
<td>Pleiotropic roles</td>
<td>AGER, TLR4</td>
<td>Secreted via conventional secretory pathways or released upon PMP</td>
<td>Not yet formally involved in liver disease, participate in hepatocellular carcinogenesis</td>
<td>[138]</td>
</tr>
<tr>
<td>S1P</td>
<td>Lipid Anti-apoptotic stimulus</td>
<td>S1PR</td>
<td>Secreted via a carrier-dependent mechanism or released upon PMP</td>
<td>Implicated in the pathogenesis of post-transplantation and conA-triggered hepatic damage</td>
<td>[153, 154]</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Purine catabolite Pot-inflammation metabolite</td>
<td>NLRP3</td>
<td>Released upon PMP</td>
<td>Implicated in acetaminophen hepatotoxicity</td>
<td>[129, 130]</td>
</tr>
</tbody>
</table>

AGER, advanced glycosylation end product-specific receptor; conA, concanavalin A; CRT, calreticulin; DAMP, damage-associated molecular pattern; ER, endoplasmic reticulum; FPR1, formyl peptide receptor 1; HBV, hepatitis B virus; HMGB1, high mobility group box 1; HSP, heat-shock protein; IL, interleukin; IL1RL1, IL receptor-like 1; IR, ischemia reperfusion; mtDNA, mitochondrial DNA; nDNA nuclear DNA; NLRP3, NOD-like receptor family, pyrin domain containing 3; NMP, nuclear membrane permeabilization; PAMPX1, pannexin 1; PMP, plasma membrane permeabilization; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; SIGLEC10, sialic acid-binding Ig-like lectin 10; TLR, Toll-like receptor.

protein HMGB1 gets massively released by the hepatocytes of patients undergoing acute liver failure [144,145] as well as in rodent models of hepatic IR [146,147], hepatitis B virus (HBV) infection [148] and concanavalin A intoxication [145,149], de facto playing a major etiological role in these settings (as demonstrated by pharmacological and genetic experiments). Upon binding to TLR9, nuclear DNA released by acetaminophen-exposed hepatocytes promotes the differentiation of hepatic stellate cells (HSCs), hence favoring the secretion of collagen and other fibrosis-associated factors [150], as well as the production of pro-inflammatory cytokines such as IL-1β and IL-18 by tissue-resident and freshly recruited immune cells [151]. The circulating levels of mitochondrial DNA and N-formyl peptides, both of which are reminiscent of the endosymbiotic origin of these organelles, are elevated in patients undergoing acute liver failure [152]. Moreover, Thr9−/− deficient mice as well as animals receiving a blocking anti-formyl peptide receptor 1 (FPR1) antibody are protected against acetaminophen-induced liver injury [152]. In rats, the inhibition of sphingosine kinase 2, one of the enzymes that generate the pro-inflammatory ceramide derivative S1P [131], has been shown to suppress inflammation and to attenuate hepatic damage upon transplantation [153]. Conversely, chemical agonists of the S1P receptor reportedly protect mice from concanavalin A hepatotoxicity [154]. Along similar lines, several potentially immunogenic HSPs, such as HSPA1A and HSPB1 [135,136], have been implicated in the hepatic response to IR and other hepatotoxic challenges, yet the major role of these proteins in this context seems to relate to their capacity to exert cytoprotective effects by operating as intracellular chaperones [155,156]. Furthermore, the circulating levels of IL-33 are elevated in patients...
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**Endogenous control of inflammation**

Besides the removal of apoptotic corpses and necrotic cells by phagocytes, which limits inflammation as it reduces the availability of DAMPs [160], two additional mechanisms have been shown to operate an endogenous control over hepatic inflammation, namely, cellular senescence and autophagy.

**Senescence control**

The accumulation of senescent HSCs is one of the hallmarks of some forms of fibrotic liver injury [161]. In response to inflammatory stimuli, HSCs proliferate in response to TLR4-mediated signals and to TGFβ produced by Kupffer cells [162]. However, as chronic inflammation fails to resolve, HSCs progressively acquire a senescent phenotype, hence manifesting a permanent cell cycle arrest as well as an increased secretion of specific cytokines (e.g., IL-6, IL-8, IL-11) and extracellular matrix-degrading enzymes [161]. Moreover, the expression of high levels of natural killer (NK)-cell activating receptor ligands (e.g., MICA, ULBP2, PVR) on the surface of senescent HSCs for allows their elimination by tissue-resident NK cells, an immunosurveillance mechanism that contributes to the resolution of fibrosis [161].

Although this pathway associating hepatic fibrosis, senescence, and immunosurveillance has been primarily described in a model of acute liver intoxication, the immune system may similarly be involved in the development of severe or chronic liver pathologies such as cirrhosis and hepatocellular carcinoma, especially in the context of immunosuppressed and/or aged patients [163].

**Autophagic control**

In hepatocytes, autophagy contributes to the removal of damaged (and hence potentially toxic) mitochondria [33] as well as to the control of the intracellular lipidome [164]. The selective autophagic degradation of mitochondria (also known as mitophagy) can be initiated by MOMP and often involves a well-characterized signal transduction pathway centered on the interaction between parkin and PTEN-induced putative kinase 1 (PINK1), two proteins that are mutated in familial Parkinson's disease [33]. By preserving the functional pool of mitochondria, autophagy minimizes ROS generation, hence quenching the activation of intracellular pro-inflammatory factors including the NLRP3 inflammasome and NF-κB [165]. Along similar lines, autophagy is required for the normal intracellular metabolism of lipids, as autophagic defects are associated with an increased accumulation of triglycerides, in vitro and in vivo [164]. Both these functions of autophagy are particularly important in the liver, as various hepatic disorders (i) are accompanied by mitochondrial dysfunction and ROS overproduction [112], and/or (ii) are associated with the accumulation of lipids in the hepatic parenchyma [109,115]. In addition, autophagy has recently been shown to inhibit the release of mitochondrial DNA by cardiomyocytes in vivo, thus limiting the activation of the NLRP3 inflammasome and the pathological consequence of pressure overload [125]. Furthermore, HMGB1 appears to stimulate autophagy (while inhibiting cell death) upon binding to advanced glycosylation end-product-specific receptor (AGER, best known as RAGE) on the surface of pancreatic tumor cells [166]. It remains to be determined whether similar processes also occur in the liver.

**Conclusions and perspectives**

The incidence of hepatic pathologies in developed countries is constantly increasing owing to multiple lifestyle factors (e.g., excessive caloric intake, unbalanced diet, lack of physical exercise, abuse of hepatotoxic drugs) coupled to an augmented life expectancy. Given the central role of cell death and inflammation in the etiology of most, if not all, liver disorders, one valid therapeutic strategy may involve the use of hepatoprotective and anti-inflammatory interventions, either alone or combined. Clinical
Concerning funding effective hepatoprotective measures. The inflammatory responses will lead to the development of novel and cellular actors whereby specific DAMPs modulate hepatic inflammatory responses will lead to the development of novel and effective hepatoprotective measures.

Conflict of interest
The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data
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References


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