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Crosstalk of liver immune cells and cell death mechanisms in different murine models of liver injury and its clinical relevance

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BACKGROUND: Liver inflammation or hepatitis is a result of pluripotent interactions of cell death molecules, cytokines, chemokines and the resident immune cells collectively called as microenvironment. The interplay of these inflammatory mediators and switching of immune responses during hepatotoxic, viral, drug-induced and immune cell-mediated hepatitis decide the fate of liver pathology. The present review aimed to describe the mechanisms of liver injury, its relevance to human liver pathology and insights for the future therapeutic interventions.

DATA SOURCES: The data of mouse hepatic models and relevant human liver diseases presented in this review are systematically collected from PubMed, ScienceDirect and the Web of Science databases published in English.

RESULTS: The hepatotoxic liver injury in mice induced by the metabolites of CCl₄, acetaminophen or alcohol represent necrotic cell death with activation of cytochrome pathway, formation of reactive oxygen species (ROS) and mitochondrial damage. The Fas or TNF- α induced apoptotic liver injury was dependent on activation of caspases, release of cytochrome c and apoptosome formation. The ConA-hepatitis demonstrated the involvement of TRAIL-dependent necrotic/necroptotic cell death with activation of RIPK1/3. The α -GalCer-induced liver injury was mediated by TNF- α . The LPS-induced hepatitis involved TNF- α , Fas/FasL, and perforin/granzyme cell death pathways. The MHV3 or Poly(I:C) induced liver injury was mediated by natural killer cells and TNF- α signaling. The necrotic ischemia-reperfusion liver injury was mediated by

hypoxia, ROS, and pro-inflammatory cytokines; however, necroptotic cell death was found in partial hepatectomy. The crucial role of immune cells and cell death mediators in viral hepatitis (HBV, HCV), drug-induced liver injury, non-alcoholic fatty liver disease and alcoholic liver disease in human were discussed.

CONCLUSIONS: The mouse animal models of hepatitis provide a parallel approach for the study of human liver pathology. Blocking or stimulating the pathways associated with liver cell death could unveil the novel therapeutic strategies in the management of liver diseases.

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KEY WORDS: liver immunobiology;
hepatitis;
therapy;
mode of cell death

Introduction

The liver is a pivotal organ of the body and plays a crucial role in taking up of nutrients from the gastrointestinal tract, storage of nutrients, metabolism, homeostasis, detoxification, immune regulation and tolerance, synthesis of bile, serum proteins, coagulating factors and complement proteins of the immune system. The liver is regarded as special immunological organ due to its enriched resident immune cell population like natural killer (NK) cells, NKT cells (formally called pit cells), Kupffer cells (KCs, resident macrophages of liver), dendritic cells (DCs), hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSEC), innate lymphoid cells (ILCs), B cells, T cells and cells of myeloid lineage.^[1] The ILCs are distinctly classified as ILC1s, ILC2s and ILC3s depending upon cytokine production and transcription factors involved in their development and function. ILC1s produce interferon- γ (IFN- γ) and they are dependent of transcription factor T-bet, ILC2s secrete Th2 cytokines such as IL-4, IL-5, IL-9, IL-13 and

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amphiregulin and require GATA3, and ILC3s produce IL-17 and/or IL-22 dependent on ROR γ t regulation.^[2] The liver encounters many circulating antigens and toxins of gut origin. In such a precarious milieu, the liver must have a robust immunologic mechanism to deal with constant exposure to potential insult.

The liver is an organ with dual face immunological functions. On one hand, immune reactions against harmless antigens have to be avoided (immune tolerance), but on the other hand, to combat against the hepato-tropic infectious agents (viruses, bacteria, parasites), effective immune responses have to be induced.^[3,4] The “dual edge” functions are regulated and controlled by the resident immune cells of the liver by secreting chemical mediators such as chemokines (for chemotaxis, recruitment of immune cells) and cytokines (pro-inflammatory and anti-inflammatory functions) collectively called as “microenvironment”. The pathophysiology of acute liver injury is orchestrated by the interplay of immune cells, cytokines, and parenchymal liver cells. The exacerbated immune responses following entry of antigens result in liver inflammation. Acute hepatitis is defined by liver cell death, cellular disarray and immune cells infiltration in the liver. The type of antigen, immunological reaction, cell death pathways or mode of liver cell death determines the fate of liver or immuno-pathogenesis of liver diseases (acute vs chronic) with distinct mechanisms of disease.

Animal models of hepatitis provide an excellent tool to understand the pathophysiological mechanisms and to correlate the data with clinical findings. The use of animal models in scientific research is appreciable due to mimicry with many human pathologies, easy availability of technical tools for analysis, reproducibility of data, close relevance to human parameters (physiology/metabolism), a minimal hazard to personnel and genetic deletion or insertion to study the effect of a specific gene. The animal models serve as an alternative approach for certain infectious diseases in human caused by HBV, HCV, influenza virus and HIV. Among various existent animal models of acute hepatitis, the present review summarized the mouse models of acute hepatitis with principal cellular and effective molecular players involved in liver cell death. The murine models of acute hepatitis have been categorized depending upon the nature of hepatitis inducing agent, i.e. hepatotoxin, autoimmune, immune cell dependent, Toll-like receptor (TLR) agonists, viral and fulminant hepatic models.

The hepatocytes express death receptors like Fas (CD95), TRAIL-R1 (DR4), TRAIL-R2 (DR5), TNFR1 and TNFR2 on their surface and the immune cells express death ligands like FasL, TRAIL, and TNF- α .^[5] The interaction of these death ligands and receptors in dif-

ferent liver diseases lead to liver cell death (apoptosis, necrosis or necroptosis) and it determines the outcome of a disease. Briefly, the apoptosis is a highly organized and genetically controlled type of cell death mediated by distinct extrinsic (death receptor dependent) and intrinsic (mitochondrial/caspase dependent) pathways. The key features of the apoptotic mode of cell death are membrane blebbing, shrinkage of the cell, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies.^[6] Necrosis is characterized by oncosis (swelling) and the formation of plasma membrane blebs (devoid of organelles) and rupture of the plasma membrane^[6] accompanied by a complete release of cellular constituents into the extracellular environment.

Evolving data^[5-7] have evidenced involvement of a novel cell death pathway in liver pathology termed as necroptosis. The term “programmed necrosis” or “necroptosis” was described as an alternative receptor interacting protein kinase (RIPK) mediated form of cell death initiated by necrosis factor receptors, Fas and TNF-related apoptosis inducing ligand (TRAIL).^[7] The necroptosis pathway is initiated by TNF receptors mainly dependent on RIPK1/RIPK3 activation and it is identified as “back up” cell death mechanism of apoptosis. Necroptosis is marked by cell and organelle swelling, extensive formation of intracellular vacuoles and rapid rupture of the plasma membrane.^[6,8] The execution of necroptosis starts from binding and trimerization of death ligands (TNF- α , FasL, and TRAIL) to their cognate receptors. Briefly, the downstream events of TNF-induced necroptosis are initiated by the activation/trimerization of TNF and its receptor (TNFR1) that promotes the formation of complex I (containing signaling molecules TRADD, TRAF2, TRAF5, cIAP1, cIAP2 and RIP1) and complex II (caspase-8-dependent cleavage of RIPK1 and RIPK3). Moreover, the interplay of RIPK1, caspase-8 and substrate of RIPK3 called as phosphorylated mixed lineage kinase domain-like (MLKL) defines the mode of cell death. The RIPK3-MLKL pathway (ubiquitylation) is essential to drive the necroptotic cell death while RIPK1-caspase-8 activation is required for the apoptotic cell death.^[9] However, in the absence of caspase-8, RIPK1 stimulates necroptotic cell death.^[9] It has been shown that necroptosis plays a crucial role in immune cell mediated hepatitis in mice because the inhibitors of necroptosis (i.e., necrostatin-1 and PJ34) protected liver injury in mice.^[10,11] Necrostatin-1 (Nec-1), a small tryptophan-based compound, an inhibitor of RIPK1 activity (phosphorylation), blocks the interaction between RIPK1 and RIPK3 and inhibits necroptosis. Nec-1 is widely used in cellular and animal disease models to prevent the necroptotic cell death.^[12] The present review comprehen-

Mechanisms of liver cell death and therapy

sively describes the cellular, molecular, immunological and cell death mechanisms of liver injury or hepatitis, its relevance with the human pathology and insights for novel therapeutic interventions.

Hepatotoxic murine liver injury models

Carbon tetrachloride (CCl₄) induced acute hepatitis

CCl₄ is a highly toxic liquid principally used in the manufacture of dichlorodifluoromethane, it was used as refrigerant and propellant, and it is contained in fire extinguishers and in spot removers. However, CCl₄ exposure induced acute and chronic hepatitis. In mice, a single oral administration of CCl₄ triggers acute liver injury which is a widely studied model of acute hepatotoxicity. The hepatotoxic molecule CCl₄ activates cytochromes (CYP2E1, CYP2B1 or CYP2B2) to form trichloromethyl (CCl₃) radical that reacts with oxygen to form a highly reactive oxygen species (ROS) that initiate lipid peroxidation and mitochondrial dependent liver injury and fatty degeneration.^[13] The highly reactive species named as CCl₃OO* starts lipid peroxidation and denaturation of polyunsaturated fatty acids. As a result, the mitochondrial, endoplasmic reticulum and plasma membrane permeability is lost with deregulation of Ca⁺⁺ in the cells leading to cellular demise.^[13] Additionally, CCl₄ toxicity leads to hypomethylation of cellular components and liver damage.^[13]

The increased influx of cytokines, chemokines and immune cells like neutrophils following CCl₄-induced liver injury result in hepatocyte damage (necrosis).^[14] The IL-6 deficient mice exhibited increased liver injury, inflammation, and delayed recovery following CCl₄-induced liver injury compared to wild-type mice, suggesting a protective role of IL-6 via down-regulation of matrix metalloproteinase-2 (MMP-2).^[15] The deficiency of IL-10 led to more extensive CCl₄-induced liver fibrosis and more prominent neutrophilic infiltration in IL-10 knockout mice during the acute CCl₄ challenge.^[16] The neutrophils are crucial in this hepatic model and the liver invariant NKT (iNKT) cells have known to protect CCl₄-induced hepatitis by limiting neutrophil infiltration.^[14, 17] The iNKT-deficient mice (Ja-18 knockout) are more susceptible to CCl₄-induced acute liver injury and inflammation^[17, 18] and the activation of iNKT cells by α -GalCer accelerates CCl₄-induced acute liver injury, inflammation, and fibrosis.^[18] The KCs play a vital role in CCl₄-mediated hepatitis in mice as depletion of KCs protects CCl₄-induced liver necrosis and IL-6 production.^[19] Another study^[20] demonstrated that CCl₄-mediated hepatitis was dependent upon the activity of KCs via TNF- α and FasL. The deficiency of chemokine receptor CCR6

in mice exacerbated the CCl₄-induced liver inflammation with enhanced KCs recruitment.^[21] Recently, it has been shown that chemical inhibition of c-Jun N-terminal kinase (JNK) 1 and 2 provided hepato-protection against CCl₄-mediated hepatitis in mice.^[22]

Acetaminophen/paracetamol induced acute hepatitis

Acetaminophen-induced hepatitis shares many features of hepatotoxic liver injury and administration of acetaminophen induces fulminant hepatitis with acute liver failure in mice.^[23] In this model, mechanism of liver injury (necrosis) is dependent on the accumulation of acetaminophen metabolites formed by cytochrome P450, N-acetyl-p-benzoquinone imine (NAPQI), NAPQI protein adducts, glutathione depletion, oxidative stress, and mitochondrial damage.^[24, 25] The role of JNK1 and JNK2 was elaborated in acetaminophen-mediated liver injury as the use of chemical inhibitor (SP600125) of JNK protected mice against acetaminophen hepatitis.^[22]

The inflammatory cytokines, such as TNF- α , IFN- γ , and IL-1 β are crucial for the development of acetaminophen hepatitis.^[26] The NK and NKT cells play a detrimental role in acetaminophen hepatitis as depletion of NK and NKT cell by anti-NK1.1 antibody protected mice against acetaminophen-induced liver injury.^[27, 28] The underlying liver injury was mediated by production of IFN- γ , chemokines, and up-regulation of FasL expression in the liver. A study demonstrated that the use of dimethyl sulfoxide (DMSO) to solubilize acetaminophen resulted in detrimental effect of NK and NKT cells in mice following acetaminophen hepatitis.^[29] Indeed, the DMSO activated hepatic NK and NKT cells *in vivo*, with increased NKT cell numbers and higher intracellular level of cytotoxic effector molecules like IFN- γ and granzyme B.^[29] The necroptosis or programmed necrosis is involved in acetaminophen-mediated liver injury because blockade of either RIPK1 or RIPK3 was protective in acetaminophen liver injury.^[30]

Alcohol induced acute hepatitis

The murine models of acute alcoholic hepatitis are widely used to correlate the findings with human pathology.^[31] The experimental model of ethanol-induced liver injury represents a model of acute hepatitis and predominantly depends upon apoptotic liver damage.^[32] Increased gut permeability to endotoxin leads to hypoxia-dependent liver injury^[33] with induction of CYP2E1, cytochrome P450 isoforms, formation of ROS and lipid peroxides.^[34] Furthermore, the release of pro-apoptotic factors such as cytochrome c into the cytosol and caspase activation leads to apoptotic liver injury in this model.^[34] The immune molecules play an important role in alco-

holc hepatitis as the alcohol-mediated hepatitis is associated with up-regulation of hepatic TLR9 and TNF- α activation.^[35] The IL-22 was shown to induce hepatoprotective effects in alcohol-induced hepatotoxicity in mice with decrease in inflammation and promotion of anti-apoptotic activities.^[36]

Cell death ligand mediated murine hepatic models

Anti-Jo2/FasL induced acute hepatitis

The liver is very sensitive to Fas mediated apoptosis because Fas receptor is constitutively expressed on hepatocytes. When mice are injected with anti-Fas antibody, death due to fulminant hepatitis and acute liver failure follows.^[37, 38] The administration of anti-Fas antibody rapidly induces severe damage to the liver (hepatocytes and sinusoidal endothelial cells) via massive apoptosis, indicating this animal model can be used in investigating human fulminant hepatitis.^[37] The mode of apoptotic liver injury in this model largely depends upon activation of caspase-3 and caspase-7, release of cytochrome c and apoptosome formation.^[39] Following injection of Jo2, it directly binds to Fas receptor in liver with CXC chemokine formation and inflammation dependent on caspases/mitochondrial damage and the transcription factor activator protein 1 (AP-1).^[40, 41]

TNF- α /D-galactosamine (D-GalN) induced acute hepatitis

Acute hepatitis induced by administration of TNF- α /D-GalN represents an apoptotic model of hepatitis and death of mice occurs due to enhanced systemic shock and liver insufficiency. Indeed, the amino sugar GalN sensitizes the host when it is metabolized in the liver and results in selective depletion of uridine nucleotides, which specifically inhibits transcription at hepatocyte level.^[42] Deficiency of TNFR1 in mice makes them resistant to TNF- α /D-GalN treatment, demonstrating an essential role for TNFR1 in this apoptosis model.^[42] However, the TNFR2 deficient mice are more susceptible to TNF- α /D-GalN-induced liver injury suggesting that in the absence of TNFR2, more TNF- α is available to bind TNFR1 to enhance apoptosis.^[42] The toxicity in the murine TNF- α model resembles viral form of acute hepatic failure in patients characterized by massive hepatocyte apoptosis via engagement of TNF receptors and downstream caspase-dependent liver injury.^[25, 43]

Immune cell mediated murine hepatic models

Concanavalin A (ConA)-induced acute hepatitis

ConA-induced hepatitis is a T-cell driven liver injury model and its features resemble with viral or autoimmune hepatitis in human.^[35, 44, 45] ConA is a lectin, isolated from jack bean (*Canavalia brasiliensis*), it binds to mannose residues (α -D-mannoside, methyl- α -D-mannopyranoside, α -D-glucose, and methyl- α -D-glucose) of different glycoproteins and thereby activates lymphocytes. The ConA-induced murine hepatic model was first developed by Tiegs and colleagues in 1992.^[44] Upon a single intravenous injection to mice, ConA induces acute liver damage within 8 hours in a dose range from 10-25 mg/kg. It has been shown that 15 minutes after intravenous administration of ConA, it binds to LSEC, KCs, CD4+ T cells and NKT cells to induce inflammation and hepatocyte damage.^[46] ConA induces hepatocyte death by stimulation of CD4+ T cells, NKT cells and KCs in liver, resulting in secretion of copious amounts of pro-inflammatory cytokines like TNF- α , IFN- γ , IL-6, IL-12, IL-18, and chemokines.^[35, 44, 45] In addition, the IL-10 is considered to be an anti-inflammatory cytokine and have a protective effect in this model.^[47]

The necrotic death of hepatocytes induced by ConA is accompanied by release of the aminotransferases (ALT and AST) from the cytoplasm of hepatocytes into the blood, inflammatory infiltration into the liver consisting of neutrophils, macrophages and T cells.^[35, 44, 45] The cytotoxic effector molecules and their receptors play a key role in the development of ConA-induced liver cell death. Among these molecules, the perforin-granzyme system is essential for ConA hepatitis along with induction of intracellular adhesion molecules as well as influx of IFN- γ .^[48] In ConA-induced hepatitis, the production of large amounts of IFN- γ by activated T cells is essential for liver injury,^[35, 45] and ConA hepatitis is suppressed in IFN- γ deficient mice, explaining its critical contribution in this model.^[49] A crucial role for TNF- α and FasL/Fas in ConA hepatitis is evident from previous studies^[50] because anti-TNF- α antibodies inhibited hepatitis in this model.^[51]

Evolving data has implied the role of TRAIL and its receptor (death receptor 5, DR5) in liver diseases in mice by the use of recombinant TRAIL or agonist anti-DR5-antibody in murine hepatitis.^[52-54] The TRAIL is expressed by myeloid or lymphoid immune cells in the liver like NK and NKT cells while DR5 expression is mainly found on hepatocytes. Expression of TRAIL and DR5 is increased following ConA hepatitis evidencing a critical contribution of these molecules to the development of hepatitis. The study^[52] demonstrated the critical role of TRAIL in ConA hepatitis because liver cell death was suppressed in TRAIL-knockout mice or by blocking of DR5 receptor. In human, TRAIL interacts with four receptors i.e. TRAIL-R1 (death receptor 4), TRAIL-R2

(death receptor 5, KILLER, or TRICK-2), TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), however, TRAIL-R1 and TRAIL-R2 induce apoptosis while TRAIL-R3 and TRAIL-R4 could not induce apoptosis.^[55, 56] In mice, only one TRAIL receptor (DR5) has been identified that shares homology with human DR5 or TRAIL-R2 and activates cell death.^[57] The STAT4 was proved to be hepatoprotective during ConA hepatitis in mice as genetic ablation of STAT4 in mice exhibited enhanced liver injury.^[58]

During ConA hepatitis, the downstream cascade of cell death signaling and adaptor protein complexes after engagement of cell death ligands and receptors result in necrotic and necroptotic liver cell death. Recent findings demonstrated the involvement of necroptotic cell death pathway in ConA hepatitis and pre-treatment of mice with Nec-1 and PJ34 (inhibitors of RIPK1 and PARP-1) ameliorated ConA-induced liver injury.^[10, 11] These studies suggested that inhibition of necroptotic cell death pathway may have therapeutic potential in the treatment of immune cell mediated hepatitis.

α -Galactosylceramide (α -GalCer)-induced acute hepatitis

The α -GalCer is a glycolipid, originally isolated from a marine sponge (*Sphingomonas microorganism*), which specifically activates iNKT cells (V α 14 NKT cells) via antigen presenting cells in the context of CD1d. The α -GalCer-induced hepatitis is associated with up-regulated expression of pro-inflammatory cytokines like TNF- α , IFN- γ , IL-2, IL-4, and IL-6 produced by activated NKT cells in the liver.^[59, 60] However, the acute liver injury induced by α -GalCer administration in mice is mediated by TNF- α and independent of KCs.^[60] Pre-treatment of mice with D-GalN exacerbated the α -GalCer mediated hepatitis in mice with massive parenchymal hemorrhage, hepatocyte apoptosis and sinusoidal endothelial cell injury.^[61] Recently, it is reported that the liver iNKT cells produce IL-17 in response to α -GalCer stimulation which induce protective effect on α -GalCer liver injury.^[62] Depletion of IL-17 by neutralizing antibodies aggravates the α -GalCer-induced liver injury, with increased hepatic neutrophil and monocyte infiltration.^[62] The administration of recombinant IL-17 abolishes these effects.^[62] The role of resident hepatic B cells was dissected in α -GalCer mediated liver injury demonstrating that iNKT cells stimulation and recruitment of innate-like regulatory B cells to the liver suppressed the liver inflammation.^[63]

TLR3 agonist, Poly(I:C) induced acute hepatitis

The polyinosine-polycytidylic acid Poly(I:C) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infections. The

Poly(I:C) is a viral dsRNA mimetic, sensed by the endosomal receptor, TLR3,^[64] as well as recently discovered cytoplasmic receptors, such as RNA helicase retinoic acid-inducible gene-I (RIGI) and melanoma differentiation-associate gene 5 (MDA-5).^[65] Upon Poly(I:C) recognition by the immune cells, TLR3 activates the transcription factor interferon regulatory factor 3 (IRF3), through the adapter protein Toll-IL-1 receptor (TIR) domain containing adapter inducing IFN- α (TRIF, also known as TICAM-1).^[66] Activation of IRF3 leads to the production of type I IFN, especially IFN- β . A second pathway involves the recruitment of TNF receptor-associated factor 6 (TRAF6) or receptor interacting protein 1 (RIP1), with the subsequent activation of transcription factors NF- κ B and AP-1.^[67]

Poly(I:C) activates macrophages, NK cells, and other lymphocyte sub-population^[68] with induction of inflammatory cytokines (TNF- α , IFN- γ , IL-6, and IL-12) and Type I IFN (IFN- α and IFN- β), a similar signature found during viral hepatitis.^[64, 69, 70] The Poly(I:C) administration in mice induces acute liver injury and the liver injury becomes lethal or aggravates if mice are pre-treated with D-GalN.^[70] The NK cells mediated acute hepatitis by Poly(I:C) has been shown to be attenuated in Bruton's tyrosine kinase (Btk) knockout mice, implicating a role for this kinase in TLR3 dependent liver injury and NK cells activation in the liver.^[71] The Poly(I:C) treatment induced production of IL-17A from hepatic $\gamma\delta$ T cells which aggravated the liver injury in mice suggesting a detrimental or pathological role of IL-17 in Poly(I:C)-induced hepatitis.^[72] Our data demonstrated up-regulated expression of IL-33 during Poly(I:C) fulminant hepatitis^[73] and the hepatocyte-specific IL-33 expression was down-regulated by treatment of PJ34.^[11] Moreover, a protective role of regulatory T cells (Treg) was found in Poly(I:C)-induced hepatitis that was dependent upon production of inhibitory cytokines (TGF- β and IL-10) in the liver.^[74]

Bacterial toxin (or LPS) induced acute hepatitis

The lipopolysaccharides (LPS) are major pathogenic factors of Gram-negative bacteria that induce systemic pro-inflammatory responses culminating in multiple organ failure and death. The LPS/D-GalN-induced hepatitis in mice is a well known animal model of acute hepatic failure. In this model, the liver injury critically depends on macrophage (KCs) derived pro-inflammatory cytokines, including IL-1, IL-6, and TNF- α .^[75] The soluble TNF- α (but not membrane TNF- α) mediates LPS-induced hepatitis in mice.^[76] The D-GalN/LPS induced liver injury was dependent on neutrophil activation and TNF- α production which caused hepatocyte necro-

sis and organ failure.^[77] Other studies had shown that TNF- α mediated caspase-dependent hepatocyte apoptosis as a predominant mechanism of LPS associated liver injury.^[75, 78] The hepatocyte apoptosis in D-GalN/LPS induced hepatitis is mediated not only by TNF- α and Fas/FasL cytotoxicity but also involves a perforin/granzyme cell death pathway.^[79] Interestingly, the activation of TLR3 ligand by Poly(I:C) attenuated the LPS/D-GalN-induced fulminant hepatitis by down-regulation of TLR4 expression in liver macrophages (KCs).^[75] The bacterial toxin such as *Pseudomonas aeruginosa* exotoxin A (PEA) induces liver injury via activation of NK cells, T cells and KCs in association with increased expression of IFN- γ , TNF- α , IL-18 and perforin.^[80, 81] Recent studies showed that the LPS/D-GalN-induced liver injury employed the hepatocyte intrinsic TNFR1 pathway in mice following secretion of TNF- α by activated KCs in liver.^[82, 83] The cytokine IL-17A played a regulatory role in neutrophil induced liver injury following LPS/D-GalN injection as the inflammatory response was decreased and the survival rate was increased in IL-17A deficient mice compared to wild-type mice.^[84] The pharmacological inhibition of phosphoinositide 3-kinase (PI3K) led to hepato-protection in LPS-injected mice by suppressing the phosphorylation of I κ B.^[85]

Mouse hepatitis virus type 3 (MHV3) induced acute hepatitis

The coronaviruses, including MHV are large, enveloped, positive-strand RNA viruses, with genome ranging in size from 27-32 kb. The hepatotropic MHV3 serotype induced severe fulminant hepatitis in mice with lethality depending upon virus strain, route of infection, age, genetic background and immune status of the mice.^[86] Several strains of MHV induce acute encephalitis and acute and chronic demyelinating disease in mice.^[86, 87] The MHV-induced hepatitis is an excellent model for studying the immunological disorders associated with viral hepatitis and it has mimicry with human HBV infection.

The MHV interacts with a specific receptor called carcino embryonic cell adhesion antigen 1 (CEACAM1) which is expressed by the hepatocytes, LSEC, NK cells, and KCs.^[88] The MHV3 (pathogenic strain L2-MHV3) can replicate in the hepatocytes, LSEC and KCs, leading to virus induced necrotic cell death.^[88] The pathogenic L2-MHV3 virus is a cloned sub-strain isolated from the liver of infected DBA2 mice and propagated in L2 cells (continuous mouse fibroblast L2 cell line). The pathogenic L2-MHV3 virus induces fulminant hepatitis via activation of NK cells in susceptible C57BL/6 mice and their death within 3-5 days post-infection with extensive necrosis in the liver and immunodeficiency in

several lymphoid organs.^[88-90] Another pathogenic strain, MHV-A59 (derived from normal mouse liver cell line-NCTC-1469 or from L2 cells) induced fulminant hepatitis as well as autoimmunity in susceptible C57BL/6 mice with formation of autoantibodies (autoAb) to fumarylacetoacetate hydrolase (FAH), a soluble cytosolic enzyme present in the liver and kidneys.^[91] The acute liver injury (after 3-5 days infection) induced by MHV-A59 is associated with an increase level of IL-6, TNF- α and IL-17 in mice.^[89] In contrast, the non-pathogenic strain isolated from persistently infected YAC lymphoid cell line (YAC-MHV3) does not induce an acute lethal disease but only subclinical infection in mice.^[92] Recent data evidenced the invasion of MHV in the brain microvasculature by impairment of IFN- β production^[93] or by sustained CXCL1 expression.^[94] The pathogenic infection by MHV3 in mice up-regulated expression of IL-33 in the liver along with other pro-inflammatory cytokines such as IL-6, TNF- α , IL-1 β , and IFN- γ .^[73]

In summary, the hepatotoxic agents such as CCl₄, acetaminophen, alcohol or their metabolites, the viral infectious agent (MHV3) or TLR-mimetic LPS or Poly(I:C), the death ligands (Fas, TRAIL or TNF- α) and the immune-cells activating hepato-tropic agents like ConA/ α -GalCer interact with specific resident immune cells in the liver. The interplay of invading hepato-tropic antigens with NK cells, NKT cells, ILCs, KCs, LSECs, vascular endothelial cells (VECs), and DCs results in release of cytokine or inflammatory mediators that lead to liver injury via apoptosis, necrosis or necroptosis (Fig. 1) depending upon the agent involved (Table). The liver cell death following receptor-mediated recognition of hepatotropic agents is initiated by the engagement of cell death ligands such as Fas, TRAIL, and TNF- α with their cognate receptors FasR, TRAIL-R, and TNF-R, respectively. The cell death signaling is represented by the formation of cell death platforms called as complex-I and complex-II importantly comprising of adaptor molecules such as FADD, RIPK1, RIPK3 caspase-8, and TRAD. The downstream signaling is the execution of mode of cell death by the action of caspases (apoptosis), RIPK1-FADD-TRAD pathway (necrosis) or RIPK1-RIPK3-FADD pathway (necroptosis) with subsequent activation of transcription factors (Fig. 2).

Surgical models of mouse hepatitis and mode of liver cell death

The necrotic mode of cell death is implicated during surgically induced liver injury such as ischemia-reperfusion (I/R), bile duct ligation (BDL) and partial hepatectomy.^[5, 95] During these liver injury models, hypoxia leads

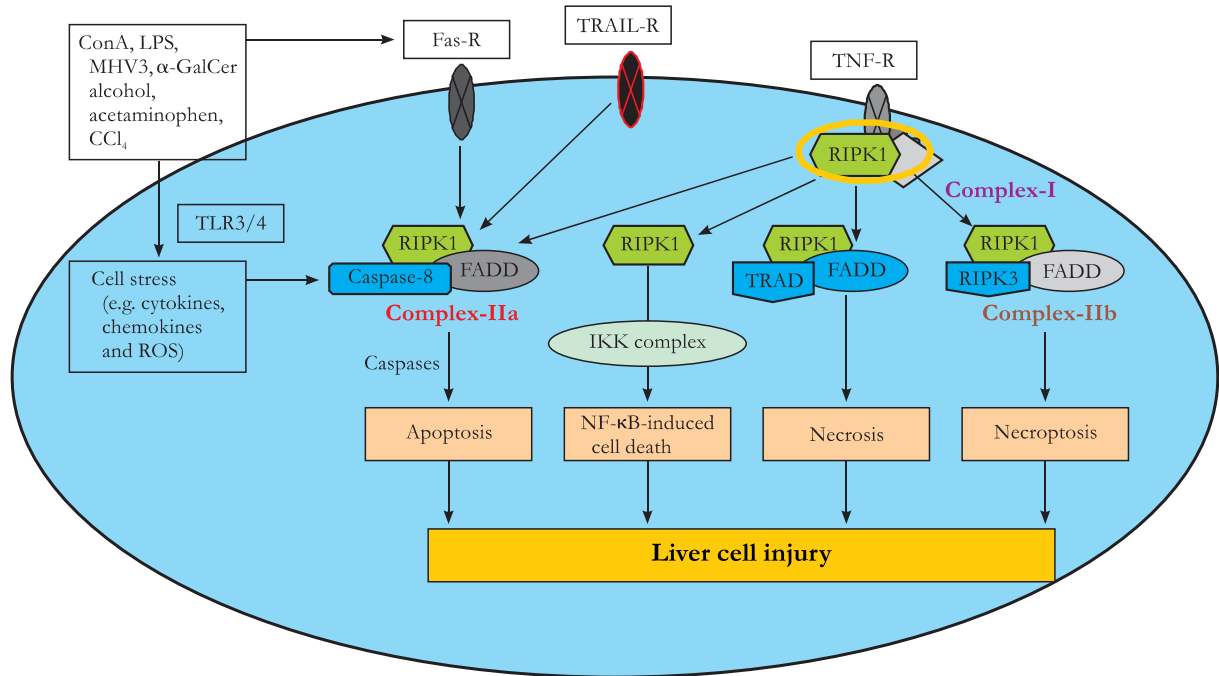


Fig. 2. Mode of liver cell death and signaling pathway. Mechanism of liver injury induced by CCl₄, acetaminophen, alcohol, ConA, α-Galcer, Poly(I:C) and LPS (endotoxins), TNF-α, TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL). Pro-inflammatory cytokines are produced by many liver cell types including KCs, NK cells, NKT cells and lymphocytes in response to inflammation, infection and other environmental stresses, which in turn cause liver injury via different modes of cell death importantly apoptosis, necrosis and necroptosis.

transplantation or liver surgery in human.

Relevance of liver cell death mechanisms with human pathology

Cell death in the liver is essentially a result of chronic derangement of liver homeostasis^[98] which accounts for a large number of chronic diseases of the liver. Programmed cell death is a key homeostatic component as it gets rid of chronically ill cells before they become malignant.^[98] The KCs secrete TNF-α, NKT cells express FasL and NK cells express TRAIL; these immune cells are principal mediators of cell death in human liver pathology.^[99] Briefly, in viral hepatitis, there is a direct relationship between apoptosis and inflammation such as in HCV infection^[100] with enhanced activation of caspases in ongoing viral inflammation.^[101] In HCV infection, NK cells are activated by type I IFN and DCs^[102] to produce TNF-α and liver damage. Apoptosis stimulated by FasL provides an efficient mean to remove unwanted HBV/HCV-infected hepatocytes and liver cancer cells by T lymphocytes.^[5] Viral hepatitis by HBV and HCV increases Fas expression on hepatocytes and TRAIL expression on NK cells for the elimination of viruses.^[103] In response to chronic liver disease, NK cells express

apoptosis-inducing mediators such as TRAIL-R2 and Fas that drive apoptosis in HBV infection in human.^[104] The TNF-α and TNFR1 have also been implicated in driving apoptosis in HCV via cytotoxic T lymphocytes.^[5, 101]

In drug-induced liver injury which is idiosyncratic drug-induced liver injury with certain haplotype human leukocyte antigen (HLA) genetic predisposition has been linked to immune-mediated apoptosis. Hapten presentation leads to activation of cytotoxic CD8 T-cells with the expression of FasL, TNF-α and to a smaller extent perforins that mediate cell death.^[105]

In non-alcoholic fatty liver disease (NAFLD), the immune cells like monocytes and macrophages play an important role in liver injury. These cells secrete inflammatory cytokines IL-6 and TNF-α to aggravate the liver damage.^[106] In severe form of NAFLD, namely non-alcoholic steatohepatitis (NASH), in a milieu of exacerbated inflammation and fibrosis, expression of death receptors such as Fas, TRAILR-2, and TNF receptor is increased.^[107] The Fas expression and infiltration of FasL-expressing cytotoxic T lymphocytes led to an apoptotic liver injury. Recent data correlated the human and murine models of NASH and demonstrated the over-expression of RIPK3 in human NASH and in a dietary mouse model of steatohepatitis.^[108] The underlying mechanism was shown to be mediated by RIPK3 and JNK necrop-

tosis signaling, the release of inflammatory mediators, liver infiltration of macrophages culminating in liver cell death and fibrosis.^[108] Studies in the past have reported the role of polymorphonuclear cells in cell death during alcoholic liver disease in human^[109, 110] with increased expression of Fas and TNFR1.^[106] However, more recent literature implicates a greater role of KCs and TNF- α as the main inflammatory mediator that activates the apoptotic pathway.^[107] The above data suggested that the cell death pathways and immune cells play a vital role in the human liver pathology and there is mimicry of mechanisms of liver disease with murine models of hepatitis.

Conclusions

The mechanisms of liver cell death and the crucial role of immune mediators in liver pathology in different animal models of hepatitis will provide the basis for the understanding of human liver disease or its relevance to clinical pathology. Modulation of immune cells-mediated liver injury and targeting of liver cell death pathways by chemical inhibitors could be promising strategies for the treatment of liver diseases. In the future, studies focusing on novel therapeutic targets or interventions in mouse hepatic models will be needed to translate the findings into clinical practice.

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