Published in final edited form as:

Semin Liver Dis. 2016 February ; 36(1): 27–36. doi:10.1055/s-0035-1571272.

Targeting Cell Death and Sterile Inflammation Loop for the Treatment of Nonalcoholic Steatohepatitis

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Abstract

Nonalcoholic fatty liver disease represents a wide spectrum of conditions and is currently the most common form of chronic liver disease affecting both adults and children in the United States and many other parts of the world. Great effort has been focused on the development of novel therapies for those patients with the more advanced forms of the disease, in particular those with nonalcoholic steatohepatitis (NASH) and liver fibrosis that can be associated with significant morbidity and mortality. In this review, the authors focus on the role of cell death and sterile inflammatory pathways as well as the self-perpetuating deleterious cycle they may trigger as novel therapeutic targets for the treatment of fibrotic NASH.

Keywords

liver disease; cell death; danger-associated molecular patterns; pattern recognition receptors; inflammation; therapy; nonalcoholic fatty liver disease; nonalcoholic steatohepatitis

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common causes of chronic liver disease worldwide. Estimates of NAFLD prevalence based on "cryptogenic" abnormal liver function, autopsy samples, and findings from ultrasonography and magnetic resonance spectroscopy are 3% to 37%, with the usual figure quoted at approximately 30% (reviewed elsewhere[1] [2]). The spectrum of NAFLD includes isolated steatosis and nonalcoholic steatohepatitis (NASH). Although patients with isolated steatosis appear to have a benign nonprogressive clinical course, those with NASH, characterized by steatosis along with hepatocellular injury, inflammation, and varying degrees of fibrosis[3] may have a potentially serious condition.[4] [5] Among these patients, those with liver fibrosis (stage 2 or higher) appear to be the ones at higher risk of overall and liver-related morbidity and mortality.[6] With NAFLD becoming increasingly common in the developed world over the last decade, NASH demonstrated the greatest increase as a cause of chronic liver disease among new liver-transplant waitlist registrations, increasing almost twofold and becoming

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the second leading etiology of liver disease among new liver transplant waitlist registrations in 2013.[7]

The clinical importance of NAFLD and the current lack of effective medications to stop or reverse disease progression in patients with NASH have sparked great interest and intense investigation to identify relevant pathophysiologic mechanisms that can be the target for the development of novel therapies. The current and most accepted concept outlining the pathogenesis of NAFLD involves multiple “hits.”[8] These hits are characterized by the occurrence of parallel and sequential events that are the result of a complex interaction between environmental factors, host genetics, and gut microflora and involve both intrahepatic and extrahepatic pathways.[9] [10] This interaction might promote isolated steatosis, innate immune activation, inflammation, cell death, or fibrosis with progressive liver damage.[8] Current pharmacotherapy efforts toward NASH can be largely divided into those with a predominant metabolic, antisteatotic effect such as insulin sensitizers and nuclear receptor modulators, and those with a direct anti-inflammatory, hepatoprotective effect. In this review, we focus on the latter. We present new insights into the relevance of various cell death pathways, sterile inflammation, and the crosstalk between them as key mechanisms in NASH pathobiology and progression, as well as discuss the evolving therapies that are either being tested or have significant potential for the treatment of NASH in patients affected with the more severe forms of this condition.

Increased Cell Death and Activation of Sterile Inflammatory Pathways as a Key Self-Perpetuating Loop Involved in Liver Injury and Fibrosis in NASH

Although several of the early triggers of hepatic steatosis can be traced to events that occur outside the liver in distant organs such as the gut, adipose tissue, and muscle among others, excessive hepatocyte cell death by apoptosis, necrosis, and other forms of cell death (see below) followed by the release of danger or stressed signals by these hepatocytes, and activation of sterile inflammatory pathways can initiate an intrahepatic, self-perpetuating noxious loop that results in chronic injury and fibrosis as an intrinsic response to this damage that can eventually progress to excessive scarring and liver failure ([Fig. 1]).[11] [12]

Because the original description that caspase activation and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) positive cells are characteristic pathologic features in the liver of NASH patients,[13] growing data have demonstrated that hepatocyte cell death is a key process involved in NASH pathogenesis.[14] [15] Sustained hepatocyte cell death has also been implicated in the development of hepatic fibrosis.[16] [17] In addition to the classical modes of cell death, such as apoptosis and necrosis (oncosis), other forms of hepatic cell death have been more recently described in preclinical models and patients with NASH, including autophagic cell death, pyroptosis, and necroptosis.[18] [19] [20] Apoptosis, a highly organized and genetically controlled process, is the most investigated and best defined form of programmed cell death in NASH. Apoptosis is initiated by either membrane receptors (extrinsic pathway) or intracellular stress leading to organelle dysfunction (intrinsic pathway). Both pathways tend to converge

in the activation of effector caspases 3 and 7, which execute the final apoptotic changes.[21] Necrosis or oncosis is an accidental form of cell death, with the fatal consequence being cellular oxygen deprivation, whereby the generation of reactive oxygen species (ROS) leads to mitochondrial dysfunction and a drop in ATP level below the threshold required to maintain cellular integrity.[22] [23] The latter induces membrane rupture with the release of cellular contents.[24] [25] Although ROS production and mitochondrial dysfunction is a central feature of NASH, necrotic cell death is a rare histopathological feature of the disease. Necroptosis is induced by the same death receptors that activate the extrinsic apoptotic pathway, namely tumor necrosis factor receptor-1 (TNF-R1) and Fas.[26] Upon interaction of receptor protein kinases 1 and 3 (RIP1 and RIP3) and a deficiency or absence of caspase 8, cell death, which morphologically resembles necrosis, occurs.[27] Controversy exists on the potential role of this form of cell death in NASH. Pyroptosis is a novel, caspase 1-dependent form of programmed cell death that has been recently shown to occur in vivo during liver injury and that shares features of apoptosis such as DNA fragmentation and necrosis such as plasma membrane permeabilizaton.[20] [28] It is dependent on inflammasome-mediated caspase 1 activation and results in the formation of discretely sized ion-permeable pores in the plasma membrane, which leads to water influx and cell swelling. [29] Its potential role in NASH has yet to be explored.

Dying hepatocytes in which a particular molecular cell death pathway is activated are capable of releasing stress signaling molecules called damage-associated molecular patterns (DAMPs) that can act on neighboring cells, including other hepatocytes as well as nonparenchymal cells of the liver such as immune cells—mainly liver macrophages or Kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells—triggering a variety of responses that initiate an homeostatic, wound-healing response to repair tissue injury. However, the persistence of these signals can induce an exuberant response that can result in tissue inflammation and excessive scarring. Recent advances have occurred in the identification of several DAMPs that play a role in tissue injury during NASH development ([Table 1]). A central consequence of the release of DAMPs is the activation of a sterile inflammatory response via their interaction with immune cells that can result in a full inflammatory response in the absence of infection. Damage-associated molecular patterns are recognized by immune cells via pattern-recognition receptors (PRR). Two key families of PRR have been growingly involved in NASH pathobiology and will be the focus of this review, including membrane-bound toll-like receptors (TLRs) and the cytosolic complex termed the inflammasome.

Targeting Cell-Death Pathways

Organelle-Initiated Cell Death

There are several mitochondrial-, lysosomal-, and endoplasmic reticulum stress-related pathways that could potentially be targeted to attenuate apoptosis. It may be possible to decrease lysosomal effects by targeting the action of cathepsin B. We have already described the proteolytic effects of cathepsin B, including the activation of cell-signaling pathways ultimately leading to cell death. In a preclinical study using a mouse model of the effects of NASH, administration of a cathepsin B inhibitor (R-3020) to mice fed a high-carbohydrate

diet led to decreased lysosomal permeabilization and decreased steatosis and liver injury.[22] Both genetic and pharmacological inhibition of cathepsin B decreased hepatocyte apoptosis and histologic evidence of liver injury in a mouse model of cholestasis.[30] More recently, the reversible cathepsin B inhibitor VBY-376 demonstrated potent activity in a mouse model of liver fibrosis. Furthermore, a phase I study to evaluate the safety and pharmacokinetics of VBY-376 in humans was conducted and no serious adverse events were observed.[31] Wu et al using 18β-glycyrrhetinic acid, a biologically active metabolite of licorice root extract, demonstrated that it prevented free fatty acid-induced lipid accumulation and hepatocyte apoptosis both in vitro in a human liver cell line and in vivo in a rat NAFLD model.[32] The proposed mechanism involved stabilizing the lysosomal membrane, inhibiting cathepsin B expression and enzyme activity, and reducing mitochondrial cytochrome c release.

Death Receptors

Mediators of the extrinsic pathway are also potential therapeutic targets for patients with NASH. Blocking the activation of death receptors, such as TNF receptor 1 (TNF-R1), Fas, and tumor necrosis factor-related apoptosis-inducing ligand receptors 1 and 2 (TRAIL-R1/2), and their associated signaling cascades may lead to decrease cell death, inflammation, and fibrosis in patients with NASH. There has been active research on components of the TNF-α and TNF-R1 cascade, including the evaluation of several TNF-α inhibitors as potential therapies for NASH. Pentoxifylline, a weak TNF-α inhibitor, has been studied in several open-label trials.[33] [34] Preliminary results did demonstrate improvement in transaminases and liver histology; however, significant side effects were documented as well, including severe nausea and other gastrointestinal symptoms. More recently, a double-blind, randomized, placebo-controlled trial demonstrated the efficacy of pentoxifylline in improving alanine transaminase (ALT) and histological features of NASH, including hepatocyte apoptosis, without significant side effects.[35] Etanercept, a fusion protein that acts as a “decoy receptor” for TNF-α as well as the various anti-TNF-α monoclonal antibodies currently available for clinical use, have been proposed as potential therapy for the treatment of NASH. However, caution must be taken as a randomized placebo-controlled trial has demonstrated that etanercept was associated with a significantly higher mortality rate in patients with moderate-to-severe acute alcoholic hepatitis (Model for End-Stage Liver Disease Score≥15).[36] Future therapies may also target interrupting the Fas–FasL interaction and formation of the death-inducing signaling complex (DISC). Zou et al performed preclinical studies utilizing Tyr–Leu–Gly–Ala (YLGA) peptides, short strands of peptides corresponding to a portion of FasL that bind readily to Fas. YLGA tetramers were administered to ob/ob mice daily for up to 4 weeks. Mice injected with YLGA peptides had significant reductions in hepatic apoptosis, hepatic inflammation, and ALT levels compared with control animals.[37]

Caspases

Given the key role that caspases play in the intracellular cascade leading to apoptosis, several groups have developed caspase inhibitors as a means to decrease apoptosis. The majority of inhibitors that have been developed are pan-caspase inhibitors. The first pan-caspase inhibitor to enter clinical trials was IDN-6556; it has been studied extensively.[38]

When administered to bile-duct–ligated (BDL) mice as a model for hepatocyte apoptosis and liver fibrosis, apoptosis, bile infarcts, chemokine activation, and serum ALT levels were reduced.[30] Similar to the other caspase inhibitors described in this section, IDN-6556 not only inhibits proapoptotic caspases, but also proinflammatory caspases, in particular caspase 1, whose activation is a key consequence of the NLRP3 inflammasome response and will be reviewed below in the section devoted to sterile inflammatory cascades. Thus, part of the effects of these drugs may be related to inhibition of this alternative pathway.[39]

The pan-caspase inhibitor VX-166 was used in a mouse model of NASH (genetically obese male db/db mice fed the methionine–choline-deficient [MCD] diet). VX-166 improved liver fibrosis; however, it failed to improve ALT levels or the net liver injury, as assessed by the NAFLD activity score.[40] The mechanism for decreased fibrosis was thought to be related to the fact that phagocytosis of apoptotic hepatocytes activates HSCs and VX-166 inhibited HSC activation by apoptotic bodies. More recently, Anstee et al evaluated VX-166 in mice models of steatosis (high-fat diet) and steatohepatitis (db/db mice on MCD diet) and demonstrated that VX-166 did not reduce steatosis, but reduced histological inflammation, apoptosis, ALT levels, and oxidative stress, particularly in the MCD model.[41]

The use of caspase inhibitors in human liver disease is being explored. A multicenter, placebo-controlled trial used IDN-6556 in 105 patients with chronic liver diseases, mainly chronic hepatitis C (n = 80), and a few NASH patients (n = 5).[42] IDN-6556, given for 14 days, significantly lowered aminotransferase activity in both hepatitis C virus and NASH patients and appeared to be well tolerated. In a follow-up, larger, double-blind, randomized, placebo-controlled, parallel-dose study using the same compound also named Emricasan (Conatus Pharmaceuticals Inc.), 204 chronic hepatitis C patients were treated with placebo or Emricasan, twice daily for up to 12 weeks. They demonstrated that Emricasan significantly reduced serum aspartate transaminase (AST) and ALT levels and was well tolerated over the study period.[43] More recently, Emricasan was found to decrease liver injury, as well as fibrosis in a murine model of NASH.[44] Another caspase inhibitor, GS-9450, was evaluated in a phase II randomized, double-blind, placebo-controlled study in adult patients with NASH.[45] In this study, patients (n = 124, principally male, mean age 45 years, with body mass index > 30 kg/m^2) with biopsy-proven NASH were randomized to receive 1, 5, 10, or 40 mg GS-9450, or placebo once daily for 4 weeks.[45]

After 4 weeks of treatment, patients in the 40-mg treatment group experienced the greatest reduction in ALT and AST levels. At week 4, linear regression of ALT versus GS-9450 dose was highly significant ($p < 0.0001$), with 35% achieving ALT levels within the normal range (7–56 U/l) compared with 0% at study baseline, and 48% achieving normal levels of AST (5–40 U/l) compared with 20% at baseline. Placebo treatment showed no meaningful change for ALT or AST. In addition, the on-treatment measure of CK18 fragments declined in the 10- and 40-mg dose groups (median baseline and week 4 values were 540 and 445 U/l in the 10-mg group and 562 and 386 U/l in the 40-mg group, respectively). One of the concerns of chronic administration of an inhibitor of apoptosis is the theoretical potential for tumorigenesis. A recent study directly evaluated the potential effects of prolonged administration of the caspase inhibitor, Emricasan, for 26 weeks on carcinogenesis by using a humanized mouse model. Continuous exposure to this drug did not result in increased

gastrointestinal or liver tumors, or any other tumors, therefore suggesting that exposure to Emricasan was not carcinogenic.[46]

Kinase Activity

The mitogen-activated protein (MAP) kinase cascades are among the most extensively studied of the signaling systems that transmit stimuli from outside the cell to the nucleus. MAPKs play important roles in a variety of cell processes by controlling transcriptional or translational regulation.[47] Three major MAP kinase cascades have been well characterized in mammals, converging on ERKs, c-Jun N-terminal kinases (JNKs), and p38 MAP kinases; each consists of three classes of serine/threonine kinases, MAP kinase, MAP kinase kinase (MAPKK, also referred to as MEK), and MAPKK kinase (MAPKKK, also referred as MAP3K). In hepatocytes, prolonged phosphorylation of JNK, and especially activation of JNK2, leads to the activation of caspase-8 and the mitochondrial death pathway, which is known to be pivotal in the development of NAFLD.[48] Mice lacking the antiapoptotic caspase-8 homolog cellular FLICE-inhibitory protein developed c-Jun N-terminal kinase-(JNK-) dependent liver injury 21 days after streptozotocin treatment, resulting in loss of insulin.[49] Substitution of insulin and inhibition of JNK using the SP600125 compound in vivo or the genetic deletion of JNK2 in all tissues abolished the injurious effect.[49] Hepatocyte-free cholesterol, which accumulates in NASH, but not in simple steatosis, increases LDH leakage, apoptosis and necrosis associated with JNK1 activation (c-Jun phosphorylation), mitochondrial membrane pore transition, cytochrome c release, oxidative stress (GSSG:GSH ratio) and ATP depletion. A study from the Farrell group demonstrated that $Jnk1^{-/-}$ hepatocytes are refractory to free-cholesterol-induced lipotoxicity and JNK inhibitors (1–2 μM CC-401, CC-930) blocked hepatocellular apoptosis and necrosis.[50] The mixed lineage kinases (MLKs) are a family of serine/threonine protein kinases that function in a phospho-relay module to control the activity of specific MAPKs. Members of the family include MLK1, MLK2, MLK3, dual leucine zipper-bearing kinase, and leucine zipper-bearing kinase.[51] MLK3 is one of the MAP3K that mediates JNK activation in the liver.[52] Loss of MLK3 in mice is protective against diet-induced NASH through attenuation of JNK activation.[53] Inhibitors of MLK3 showed great promise for the treatment of Parkinson’s disease in preclinical models and are a potential therapeutic target for the treatment of human NASH.[54] Apoptosis signal–regulating kinase 1 (ASK1), a 160-kDa serine/threonine protein kinase, is another member of the MAP3K family; it activates both p38 and JNK pathways.[55] ASK1 is preferentially activated in response to various types of stress such as oxidative stress and plays pivotal roles in a wide variety of cellular responses.[56] ASK1-deficient mice exhibit reduced diet-induced hepatic steatosis and fibrosis.[57] ASK1 has also been reported to be involved in liver injury induced by acetaminophen or troglitazone, a first-generation thiazolidinedione insulin sensitizer.[58] [59] In animal models of kidney disease, the ASK1 inhibitor GS-4997 improved kidney histopathologic scores and functional readouts such as direct measurements of the glomerular filtration rate, serum creatinine, and proteinuria.[60] Based on this results, phase 2 trials have been initiated in patients with NASH, pulmonary arterial hypertension, and diabetic kidney disease.[61] [62] [63] Receptor-interacting protein kinases are a group of threonine/serine protein kinases with a relatively conserved kinase domain, but distinct nonkinase regions.[64] Necroptosis is a recently described caspase 8-independent method of

cell death that denotes organized cellular necrosis and requires the coactivation of RIP1 and RIP3 kinases. RIP1 is necessary in APAP-induced liver injury and RIP1 inhibition via nectrostatin-1 (Nec-1) reduced hepatotoxicity in APAP-induced acute liver injury.[65] [66] Further, a recent report suggested that selective inhibition of RIP3 using the anticancer drug dabrafenib alleviates APAP injury.[67] The Luedde group demonstrated that RIP3 is upregulated in human NASH and in a dietary mouse model of steatohepatitis. RIP3 mediated liver injury, inflammation, the induction of hepatic progenitor cells/activated cholangiocytes, and liver fibrosis.[68] By screening conventional small-molecule libraries, three RIP3 inhibitors have been identified—GSK840, GSK843, and GSK872—which selectively inhibit RIP3 kinase-dependent necroptosis.[69] Highlighting the fact that diverse modes of acute liver injury have differing requirements for RIP1 and RIP3, a recent study showed that in ConA-induced autoimmune hepatitis, RIP3 deletion was protective, whereas RIP1 inhibition exacerbated disease, while conversely, in acetaminophen-mediated liver injury, blockade of either RIP1 or RIP3 was protective.[70] Future studies are warranted to test applicability and efficacy of the many kinase inhibitors, already available and currently under development, in patients with NASH.

Targeting Danger Signals and Their Surface Receptors

Danger signals are intracellular molecules that are released into the extracellular environment during cellular stress and death, and activate innate immune cells resulting in an inflammatory response.[71] [72] As this occurs in the absence of pathogens it is frequently referred to as a sterile inflammatory response, and the molecules are generically referred to as damage-associated molecular patterns (DAMPs), although many do not have a patterned structure.[73] [Table 1] shows the best characterized DAMPs; many more are likely involved. This deceptively simple concept results in a very complex biology. For example, the nuclear protein HMGB1 upon release can bind one of several receptors, including RAGE (receptor for advanced glycation endproducts), TLR4, and CD24—each resulting in different functions.[74] [75] [76] [77] The consequence of HMGB1-receptor binding is also dependent on the oxidation state of one of three cysteine residues. Fully reduced HMGB1 binds to $CXCR_4$ and stimulates immune cell infiltration, partially reduced HMGB1 activates immune cells to produce cytokines and chemokines via TLR4, and fully oxidized HMGB1 is devoid of immunostimulatory activity.[78]

The concept of DAMPs driving inflammation after tissue injury has been confirmed after diverse forms of injury to a wide range of organs. In the liver, a role for DAMPs has been shown in experimental injury initiated by ischemia reperfusion, acetaminophen, and alcoholic hepatitis.[79] [80] [81] [82] In addition to causing liver injury, the size of the liver and the ability of liver injury to release significant amounts of DAMPs into the circulation opens up the possibility that liver-derived DAMPs may be responsible for systemic inflammation and organ failure. It is interesting to note that many of the receptors activated by DAMPs are also activated by pathogen-associated molecular patterns (PAMPs), and include molecules in the TLR family.[83] Others such as P2 × 7 appear uniquely responsive to DAMPs.[84]

The presence of a large number of DAMPs and receptors suggests significant redundancy, which theoretically makes them unattractive candidates for therapy. However, experimental data suggest otherwise. Interference with individual DAMP pathways results in significant reduction in liver injury in a variety of models. Much of the evidence for a role of DAMPs in NASH comes from receptor-deficient mice, but this is only partially informative because many receptors have DAMPs and PAMPs as ligands. For example, mice deficient in TLR4 and TLR9 have reduced steatosis and inflammation in experimental models of NASH.[85] [86] The long-term nature of NASH models makes it challenging to test therapeutic agents, and there is very little information on direct targeting of DAMP pathways in NASH. The clinical development of antagonists of TLR4 and TLR9 for sepsis and systemic lupus erythematosus has resulted in agents that are safe, and are candidate therapeutic agents for human NASH. Eritoran (Eisai Co.) is a TLR4 antagonist that has in vivo efficacy in blocking TLR4, and protects against liver ischemia reperfusion injury.[87] IRS954 is an oligomer-based TLR9 antagonist that has undergone phase 1b clinical trials in systemic lupus erythematosus, and is a candidate for therapy in NASH.[88] In high-fat diet (HFD) models of NASH IRS954, given concurrently with HFD and 6 weeks after starting a HFD, resulted in a significant reduction in steatosis, and inflammation.[89] The reduction in steatosis is interesting, and was also seen in the original data from MCD-induced NASH in TLR9-deficient mice.[85] This suggests that either blocking TLR9 on hepatocytes or switching off the inflammatory response in liver immune cells results in reduced steatosis. This could be due to the removal of the ability of proinflammatory cytokines such as IL-1β to induce hepatocyte steatosis. The normal phenotype of TLR9-deficient mice and their lack of increased susceptibility to infections are reassuring for anti-TLR9 therapy being low risk strategy. A different approach is to use natural agents such as γ-tocotrienol, which has several effects including antioxidant, and stimulating autophagy as well as reducing cytochrome c signaling.

Overall targeting DAMP pathways in NASH is a very attractive and underdeveloped field. Antibody-based approaches have been used successfully in many types of tissue inflammation and are predicted to be effective in NASH. Inhibition of Hsp90 (heat shock protein 90) by an antibody or small molecule approach as a treatment strategy could be useful in the treatment of inflammatory diseases, including rheumatoid arthritis.[90] Inhibitors of Hsp90 blocks activation of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway, leading to a loss of cytokine production. Following Hsp90 inhibition, RIP and IκB kinase, which are members of the NF-κB signaling pathway, are degraded, and activation of MAP kinases can be blocked.[91] Interleukin-1 receptor–associated kinase is also a client of Hsp90, and consequently, inhibition of Hsp also has the ability to diminish innate immunity responses via TLR signaling. SGT1, a component of the inflammasome, has also recently been reported to be degraded following Hsp inhibition. A small molecule inhibitor of Hsp90 (SNX-7081) blocks NF-κB translocation to the nucleus and production of interleukin-1β (IL-1β) and TNFα, both of which are known to be important in NASH.[92] In models of arthritis, SNX-7081 resulted in less tissue inflammation and less overall weight loss.

The advantages of targeting DAMPs are that they are specific to tissue damage-associated inflammation, and that this is not expected to comprise the response to pathogen-derived

signals. Furthermore, DAMPs also stimulate fibrogenic responses, and there may be independent antifibrotic effects of DAMP-based therapy.

Targeting Cytosolic Receptors

In addition to cell-surface receptors, the innate immune responses to tissue injury caused by pathogens, cellular stress or environmental insults may be initiated by the cytosolic nucleotide-binding domain and leucin-rich repeat receptors (NLRs), retinoic acid-inducible gene (RIG) like helicase (RLH) receptors and absent-in-melanoma- (AIM-) like receptors.[93] The following paragraph focuses on the role of NLRs in the development and treatment of NASH.

Some NLRs form inflammasomes, which act as scaffolding protein within the complex, caspase-1 and in some cases an adaptor protein.[93] To date, the NLR family comprises 14 NLR genes identified in human and 20 in mouse. Within the liver, inflammasomes are expressed in both parenchymal and nonparenchymal cells and serve as key regulators of inflammation and cell fate.[94] [95] The most studied inflammasome, NLRP3, assembles a complex comprised of the adaptor protein apoptosis-associated speck-like protein (ASC) and the serine protease caspase-1. The NLRP3 inflammasome responds to cellular danger signals by activating caspase-1, releasing IL-1β and IL-18, as well as initiating a novel pathway triggering programmed cell death termed pyroptosis.[96] [97] [98] Activation of the NLRP3 inflammasome in mice results in severe liver inflammation, fibrosis, and hepatocyte pyroptotic cell death.[20] [99] In murine models of NASH, NLRP3 activation is required for the fibrotic response, suggesting that targeting this complex may be a rational strategy to block or reverse the development of fibrotic NASH.[76] Among the various cytokines participating in chronic hepatic inflammation, IL-1β plays a special role. IL-1β was found to promote hepatic stellate cell proliferation, activation, and transdifferentiation into a myofibroblast phenotype.[100] The therapeutic strategy to reduce NLRP3 inflammasome activity can be separated in compounds that affect NLRP3 inflammasome assembly and activation, caspase 1 activation, and IL-1 and IL-18 pathways.

The IL-1 family consists of two IL-1 receptors (IL-1R)—IL-1RI and IL-1RII—and the IL-1R antagonist (IL-1Ra), which does not have agonistic activity and does not trigger downstream signaling.[101] Therefore, anti-IL-1 therapies are based on either recombinant IL-1Ra—anakinra; or monoclonal anti-IL-1β antibodies—canakinumab; or an IL-1 trap—rilonacept. A comprehensive study by Petrasek et al demonstrated that IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice.[102] Based on these results, a phase II study was initiated to test the efficacy of anakinra, pentoxifylline, and zinc compared with methylprednisolone in severe acute alcoholic hepatitis.[103] Programmed cell death, the hallmark of NASH contributes to liver injury and fibrosis, is executed by numerous caspases. As outlined above, several studies provide strong evidence that treatment with pan-caspase inhibitors reduce liver injury and fibrosis in murine models of NASH as well as in patients.[40] [41] [44] [45] To date, only very few drugs have been described targeting the inflammasome directly; no study has evaluated applicability and efficacy of inflammasome blockade in models of NASH. A study by Coll et al identified CRID3—a member of the class of diarylsulfonylurea containing compounds called cytokine

release inhibitory drugs (CRIDs)—as a novel inhibitor of the NLRP3 and AIM2 inflammasomes.[104] Changes in the preparation of CRID3 led to the development of MCC950, a potent, selective, small-molecule inhibitor of NLRP3. MCC950 blocked canonical and noncanonical NLRP3 activation at nanomolar concentrations.[105] MCC950 specifically inhibited activation of NLRP3, but not the AIM2, NLRC4, or NLRP1 inflammasomes. MCC950 reduced interleukin-1b (IL-1b) production in vivo and attenuated the severity of experimental autoimmune encephalomyelitis (EAE), a disease model of multiple sclerosis.[105] Furthermore, MCC950 treatment rescued neonatal lethality in a mouse model of CAPS and was active in ex vivo samples from individuals with Muckle–Wells syndrome.[105] A recent study from our group using two murine models of NASH suggested that MCC950 is a potential novel therapy for this disease.[106] Future studies are warranted to decipher the role of targeting cytosolic receptors in NASH treatment.

Acknowledgments

This work was funded by NIH grants R01 DK082451 and U01 AA022489 to Ariel E. Feldstein. Alexander Wree is supported by the START-Program of the Faculty of Medicine, RWTH Aachen.

Abbreviations

AIM	absent in melanoma like receptors
ALT	alanine transaminase
APAP	acetaminophen
ASC	apoptosis associated speck like protein
ASK1	Apoptosis signal–regulating kinase 1
AST	aspartate transaminase
BDL	bile–duct ligated
CAPS	cryopyrin-associated periodic syndromes
CRIDs	cytokine release inhibitory drugs
DAMPs	damage-associated molecular patterns
EAE	experimental autoimmune encephalomyelitis
ERKs	extracellular-signal-regulated kinases
HFD	high-fat diet
HMGB1	high-mobility group box protein 1
Hsp90	heat shock protein 90
IL	interleukin
JNKs	c-Jun N-terminal kinases

MAP	mitogen-activated protein
MCD	methionine–choline-deficient
MLKs	mixed lineage kinases
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
Nec-1	nectrostatin-1
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLRs	nucleotide-binding domain and leucin-rich repeat receptor
PAMPs	pathogen associated molecular patterns
PRR	pattern-recognition receptors
RIG	retinoic acid-inducible gene receptor
RIP	receptor-interacting protein
RLH	RIG-like helicase receptor
ROS	reactive oxygen species
TLRs	toll-like receptors
TNF-R1	tumor necrosis factor-receptor 1
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand

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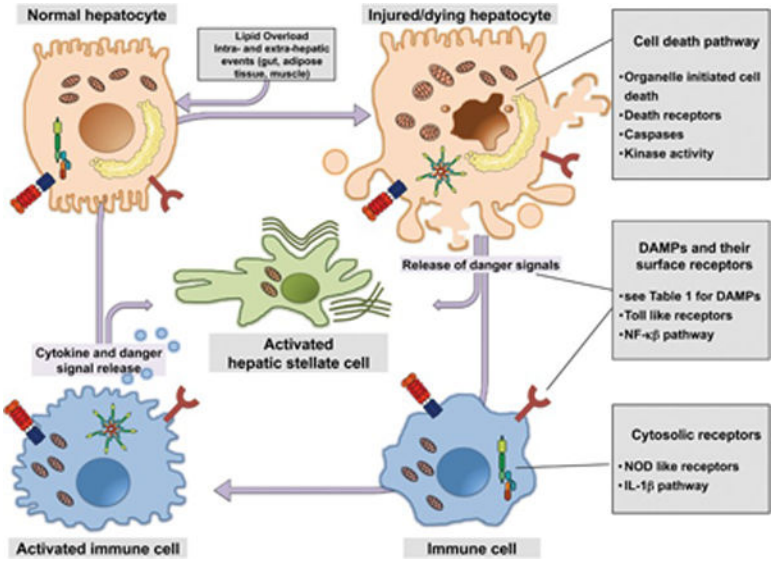

Fig. 1.

The intrahepatic self-perpetuating noxious loop in nonalcoholic steatohepatitis (NASH). Lipid overloading of the liver may result from both intra- and extrahepatic events in distant organs such as the gut, adipose tissue, and muscle, among others. Accumulation of toxic lipids in hepatocytes may induce cellular stress triggers and lead to hepatocyte cell death by various mechanisms, including apoptosis and necrosis followed by the release of intracellular molecules that act as danger signals to communicate stress to neighboring cells. These signals impart danger-associated molecular patterns (DAMPs), and activate sterile inflammatory pathways in cells of the innate immune system, which in turn release cytokines and additional danger signals. The central consequence of this chronic injury is the activation of hepatic stellate cells that can eventually progress to fibrotic NASH.

Table 1

DAMPs that may be involved in the development of nonalcoholic steatohepatitis

DAMP	Receptors	Therapy
ATP	$P2X_7$	Receptor antagonists, apyrase
Cytochrome c	Not known	γ-tocotrienol
CPS-1	Not known	Not known
Defensins	TLR4, CCR_6	Antagonists, antibodies
Fatty acids	TLR4, NLRP3	Antagonists, antibodies
Ceramides	TLR4, NLRP3	Antagonists, antibodies
Cholesterol crystals	NLRP3	Antagonists, antibodies
HMGB1	TLR4, RAGE, NLRP3	Neutralizing antibodies
HSP	TLR4, CD14, CD91	Anti-HSP antibodies
Hyaluronic acid	TLR2, TLR4	Antagonists, hyaluronidase
Mitochondrial DNA	TLR9, NLRP3,TLR9	Antagonists, DNAses
Nuclear DNA	TLR9	TLR9 antagonists, DNAses
N-formulated peptides	FPR and FPRL1	Antibodies
S100 proteins	RAGE	Blocking antibodies
Uric acid	Nonreceptor	Xanthine oxidase inhibitors

Abbreviations: ATP, adenosine triphosphate; CPS-1, carbamoyl phosphate synthetase 1; DAMPs, damage-associated molecular patterns; FPR, formyl-peptide receptor; HMGB, high-mobility group box 1 protein; HSP, heat-shock protein; NLRP, NOD-like receptor protein; RAGE, receptor for advanced glycation endproducts; TLR, toll-like receptor.