Clinics and Research in Hepatology and Gastroenterology (2015) 39, S18—S23

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MINI REVIEW

# Inflammasome activation in the liver: Focus on alcoholic and non-alcoholic steatohepatitis☆

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Available online 26 July 2015

**Summary** Upregulation of the inflammatory cascade is a major element both in the progression of steatohepatitis to severe alcoholic hepatitis as well as in the progression of NASH to advanced NASH with fibrosis. The mechanisms underpinning these changes are only partially understood. Activation of the inflammatory cascade requires multiple stimuli and in this report, we discuss the role of inflammasomes that activate IL-1β as well as the sterile and pathogen-derived danger signals that results in inflammasome activation and inflammation in alcoholic and non-alcoholic steatohepatitis. The dynamics of inflammasome activation, the cell types involved and the trigger signals appear to be somewhat different between ASH and NASH. Further studies are needed to dissect the pathology-related differences between these two major forms of steatohepatitis. Clinical and therapeutic implications of inflammasome activation in steatohepatitis are also discussed.


*Abbreviations:* ASH, alcoholic steatohepatitis; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; ALD, alcoholic liver disease; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; LPS, lipopolysaccharide; TLR, toll-like receptor; LMNCs, liver mononuclear cells; PBMCs, peripheral blood mononuclear cells; NLRs, nucleotide-binding oligomerization domain (NOD)-like receptors; NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis-associated specklike protein; AIM2, absent in melanoma 2; MCD, methionine and choline deficient; HF-HC-HS, high fat, high cholesterol, high sucrose.

☆ This article is part of the special issue ''Alcohol, Virus and Steatosis evolving to cancer'' featuring the conference papers of the 10th International Symposium organized by the Brazilian Society of Hepatology in São Paulo, Brazil, September 30th—October 1st, 2015.

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http://dx.doi.org/10.1016/j.clinre.2015.06.012
2210-7401/

## Overview of the inflammasome and inflammasome activation

The inflammasome is an intracellular multi-protein complex that plays a major role in inflammation. Inflammation is the host defense response of the innate immune system to exogenous (typically pathogen-derived) and endogenous (typically sterile) danger signals. The different danger signals are recognized by specific sensors/receptors in the pattern recognition receptor family that includes toll-like receptors, helicase receptors, Nod-like receptors and others [1]. The nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) represent the sensor component of the inflammasome [2,3]. The inflammasome also contains the adaptor protein apoptosis-associated specklike protein (ASC) and the inflammatory effector molecule caspase-1 [4,5]. Caspase-1 has enzymatic activity, which, as the effector of inflammasome activation, cleaves pro-interleukin (IL)-1β, pro-IL-18 and pro-IL-33.

Compared to other pro-inflammatory cytokines (TNF-α or IL-6) or chemokines (MCP-1) that are readily induced upon toll-like receptor (TLR) activation, IL-1β production is more tightly controlled [6]. TLR stimulation results in IL-1β mRNA production that is rapidly translated into pro-IL-1β that is not secreted from the cell until a second signal is provided that activates the inflammasome, leading to caspase-1 activation that cleaves pro-IL-1β into the mature form of IL-1β [7]. This mature, 18 kD, IL-1β is secreted from the cell into the extracellular space and acts locally or through systemic effects. Interestingly, IL-1β rapidly binds to the IL-1 receptor (IL-1R) and has strong autoregulatory feedback effects [8]. All of these have major implications in liver inflammation:

- first, due to the requirement for two signals for activation, inflammasome activation and IL-1β production might not be the initial response to a minimum inflammatory signal;
- second, because of the two signals that regulate inflammasome and IL-1β, cumulative danger signals will likely activate the inflammasome even if the signals are low amplitude such as seen in chronic inflammation states;
- third, because of its capacity to readily bind the IL-1R and autoregulatory effects, IL-1β has a major role in amplification of inflammation [6,8].

## Inflammasome activation in alcoholic liver disease

Alcoholic liver disease, and to a greater extent alcoholic hepatitis, are characterized by activation of the inflammatory cascade in the liver. Alcohol has many effects on the liver and on the different cell-types in the liver. Alcohol directly and through its metabolites damages hepatocytes, leading to hypoxia, ROS induction, and apoptosis. Through its effects on the gut, alcohol results in impaired gut barrier functions and increased gut permeability. This results in an increase in lipopolysaccharide (LPS) levels in the portal and systemic circulation. LPS is a component of Gram-negative bacteria that likely represents the most potent danger signal for innate immunity mediated via the TLR4 complex. It is plausible that the impaired gut barrier permits translocation of other bacterial components to the portal circulation resulting in activation of other TLRs.

Studies in mice found that both acute alcohol gavage and chronic alcohol feeding result in inflammasome activation and IL-1β production. Messenger RNA levels of inflammasome components, NLRP1, NLRP3, ASC, caspase-1 and pro-IL-1β were all increased in four-week chronic alcohol-fed mice compared to controls [9—11]. Functional activation of the inflammasome was demonstrated by increased cleaved caspase-1, p10, cleavage of IL-1β in the liver and increased serum IL-1β levels [10,12]. The important role of the inflammasome in alcoholic liver disease was indicated by protection of IL-1R-KO mice from alcoholic steatohepatitis. Mice deficient in caspase-1 (and -11) or the inflammasome adapter, ASC, were also protected from alcohol-induced steatosis, inflammation and liver injury [10].

It should be noted that a study by DeSantis et al. [13] suggested an opposing role for NLRP3 in a two-week 5% ethanol alcohol feeding. Compared to WT mice, NLRP3 deficiency resulted in increased serum ALT after ethanol administration. Interestingly, this study showed NLPR3-deficient mice were paradoxically protected from ethanol-induced liver TNF-α protein compared to WT mice.

Recently, our group further explored the potential role of NLRP3 in alcoholic liver disease. We found that, in a four-week ethanol feeding, mice deficient in NLRP3 were protected from ethanol-induced liver injury, inflammation and steatosis [12]. Particularly important was the absence of inflammasome activation after ethanol administration in these NLRP3-KO mice. Our data led us to reach the opposite conclusion of DeSantis et al., and strongly suggest that NLRP3 plays a critical role in inflammasome activation in alcoholic liver disease.

## Mediators of inflammasome activators in alcoholic liver disease

Our studies suggest that inflammasome activation in alcoholic liver disease occurs mostly in immune cells and not hepatocytes. Liver mononuclear cells (LMNCs) have an eight-fold higher level of basal expression of pro-IL-1β protein compared to primary hepatocytes *ex vivo*. Importantly, the heightened sensitivity in mounting an inflammasome-mediated response of LMNCs to LPS and ethanol stimulation was roughly 20-fold higher compared to primary hepatocytes [10]. While Kupffer cells, the resident macrophages in the liver, likely play the biggest role in inflammasome activity, DAMPs from ethanol-damaged hepatocytes are the greatest source of ligands.

Adenosine triphosphate (ATP) is one of several ligands capable of activating the inflammasome in the presence of LPS. ATP is released by damaged cells, triggering K+ efflux via stimulation of the ATP-gated P2X7 ion receptor [14,15]. High levels of ATP are detected in serum of chronic ethanol-fed mice [10]. Moreover, our lab recently demonstrated that purinergic signaling inhibition with either apyrase or suramin suppressed inflammasome activation in peripheral blood mononuclear cells and murine LMNCs *in vitro* [12]. Importantly, mice deficient in P2X7 had protection from liver injury, Caspase-1 cleavage and inflammation after ethanol administration. This data indicates that P2X7-mediated ATP