Review

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Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure

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Summary

Drug-induced acute liver failure carries a high morbidity and mortality rate. Acetaminophen overdose is the number one cause of acute liver failure and remains a major problem in Western medicine. Administration of N-acetyl cysteine is an effective antidote when given before the initial rise in toxicity; however, many patients present to the hospital after this stage occurs. As such, treatments which can alleviate late-stage acetaminophen-induced acute liver failure are imperative. While the initial mechanisms of toxicity are well described, a debate has recently occurred in the literature over whether there is a second phase of injury, mediated by inflammatory processes. Critical to this potential inflammatory process is the activation of caspase-1 and interleukin-1β by a molecular complex known as the inflammasome. Several different stimuli for the formation of multiple different inflammasome complexes have been identified. Formation of the NACHT, leucine-rich repeat (LRR) and pyrin (PYD) domains-containing protein 3 (Nalp3) inflammasome in particular, has directly been attributed to late-stage acetaminophen toxicity. In this review, we will discuss the mechanisms of acetaminophen-induced liver injury in mice and man with a particular focus on the role of inflammation and the inflammasome.

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Key point

Acetaminophen (APAP)-induced acute liver failure remains a major clinical problem.

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Introduction

Drug-induced liver injury and acute liver failure (ALF) remains a major problem in Western societies [1,2]. Most drug-induced liver injury and ALF occurs due to either accidental or intentional overdose of acetaminophen (APAP, paracetamol). Because of its dose-dependent toxicity, APAP-induced liver injury can be studied in animal models and in isolated hepatocytes, and most mechanisms are translatable to humans [3–5]. While significant progress has been made in the understanding of intracellular signaling mechanisms of APAP toxicity in hepatocytes, there is still a considerable debate in the literature over the role of sterile inflammation in the pathophysiology. While the presence of an inflammatory infiltrate is obvious both histologically and biochemically, whether this infiltrate directly contributes to hepatocyte death remains controversial. At the core of many of these debates lies the role of many specific inflammatory processes associated with liver injury, including the activation of the inflammasome after APAP overdose. Breakthrough studies in the early-mid 2000s first identified the presence of a highly regulated signaling system in myeloid cells that responds rapidly to the presence of damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs). This system, the inflammasome, has been extensively studied since then in the context of liver injury [6,7]. The purpose of this article is to review both recently discovered molecular mechanisms that control inflammasome activation and the role of the inflammasome in drug-induced liver injury, with a special emphasis on APAP overdose and APAP-induced ALF.

The inflammasome – A molecular mechanism for immune cell activation

Since the initial description of the activation and formation of the NACHT, leucine-rich repeat (LRR) and pyrin (PYD) domains-containing protein 3 (NALP3) inflammasome [8], there have been intensive studies on the molecular mechanisms that control the inflammasome. Ostensibly, the major purpose of the inflammasome is for immune cells to detect the presence of DAMPs and PAMPs in serum. A response is then initiated with the activa-

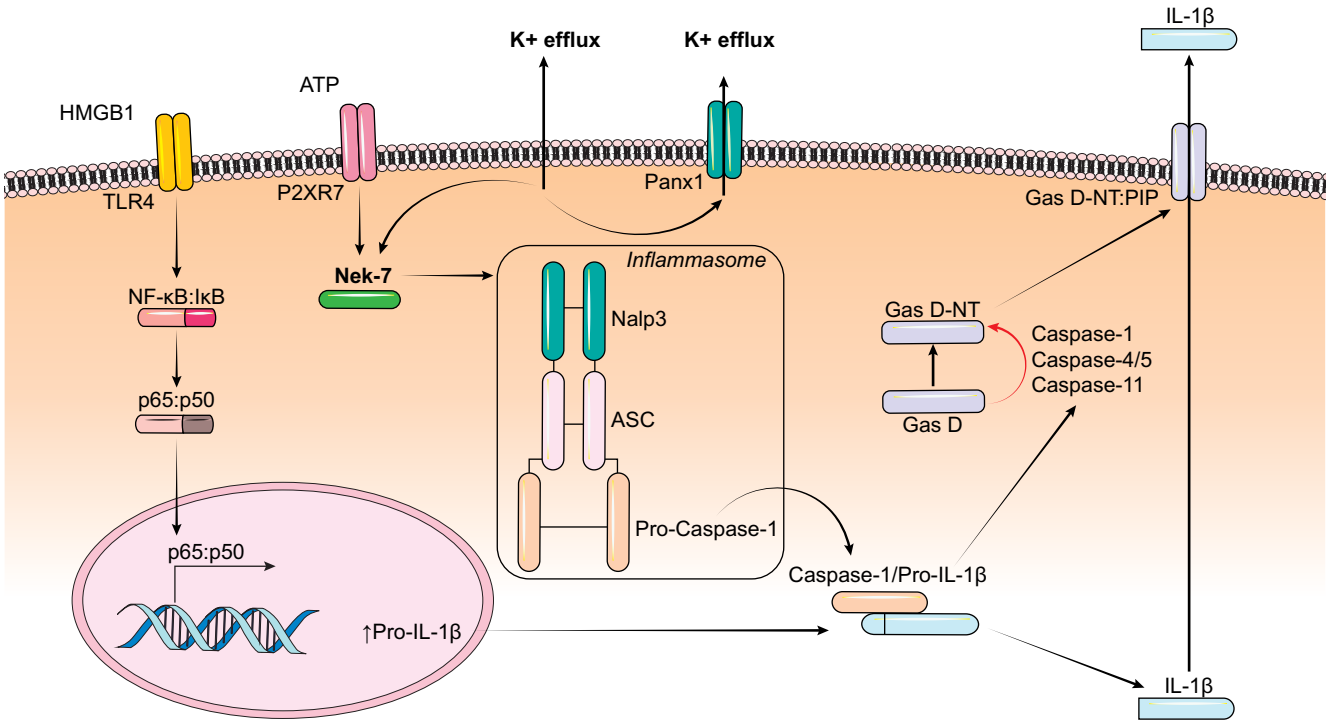

Fig. 1. Proposed mechanism of inflammasome activation by ATP: P2XR7 interaction in macrophages. Elevated ATP levels in serum released from dying cells activates P2XR7 causing pannexin-1 (Panx1) pore opening and potassium release. In addition, activation of P2XR7 causes activation of the protein Nek-7 (currently undefined function), and leads to formation of the Nalp3 inflammasome with activation of pro-caspase-1. Stimulation of Toll-like receptors, e.g., TLR4, by substrates such as high mobility group box 1 (HMGB1) protein causes NF-κB activation and transcriptional induction of pro-IL-1β formation. The active caspase-1 cleaves pro-IL-1β and the mature cytokine is released. Activation of pro-inflammatory caspases, either directly by LPS (caspase-4/5 or caspase-11), or through the inflammasome (caspase-1), results in cleavage of gasdermin D into the N-terminal (Gas D-NT) cleaved form of Gasdermin D (Gas D). The N-terminal form mediates cell death via perforation of the plasma membrane after binding plasma membrane components such as phosphatidyl inositol or cardiolipin. Pore formation results in cellular collapse in cells undergoing pyroptosis and passive release of constituents such as IL-1β. P2XR7, purinergic receptor P2XR7; Nek-7, NIMA-related kinase 7; Nalp3, NACHT, LRR and PYD domains-containing protein 3; ASC, apoptosis-associated speck-like protein containing a CARD; IL-1β, interleukin-1β; PIP, phosphatidylinositol phosphate.

tion of pro-inflammatory cytokines, interleukin-1β (IL-1β) and interleukin 18 (IL-18), through a proteolytic cleavage pathway mediated by the activation of caspase-1 [8]. IL-1β is a potent activator of effector cells such as monocytes and neutrophils that express the interleukin-1 receptor (IL-1R). As such, the commonly measured primary outcomes of inflammasome activation are increased serum levels of IL-1β and IL-18 [8] and subsequent recruitment of inflammatory cells (Fig. 1). However, the mechanism of secretion of IL-1β remains poorly defined [9]. The simplest explanation remains the idea that IL-1β is produced in cells which undergo necrosis and then release IL-1β passively [10]. This corroborates data that brefeldin A, a classical Golgi inhibitor, has no effect on IL-1β secretion [11]. Other data have supported an unconventional secretion mechanism that bypasses the endoplasmic reticulum/Golgi apparatus. This may occur independent of cell death and through mechanisms that involve autophagosomes typically associated with autophagy [12]. Vesicle and exosome release have also been implicated [13,14]. As such, it is probable that multiple mechanisms can contribute to IL-1β secretion depending on the current microenvironment and relevant cell type. The degree to which each of these contribute versus cell death via necrosis or pyroptosis has yet to be determined.

Multiple different inflammasome complexes exist (Supplementary Table 1) [6,8,15], and generally, these different pathways converge at the activation of caspase-1 and the subsequent activation of IL-1β [8,16–19]. The intracellular priming mechanisms for inflammasome activation have been studied intensely; however, no consensus activation signal has been detected [6]. Instead, a number of different extracellular signals have been defined that activate the inflammasome, which are detected by a family of proteins called nucleotide-binding oligomerization domain, leucine-rich repeat (LRR)-containing protein (NLR). These include, among others, NLRP1, NLRP3, and NLRC4 (reviewed in [6]). Each of these family members can assemble a complex with a caspase recruitment domain (CARD) via the adaptor protein, apoptosis-associated speck-like protein containing a CARD (ASC). This forms the protein complex responsible for the binding and cleavage of caspase-1