Gut–Liver Axis: Role of Inflammasomes

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Inflammasomes are large multiprotein complexes that have the ability to sense intracellular danger signals through special NOD-like receptors or NLRs. They include NLRP3, NLRC4, AIM2 and NLRP6. They are involved in recognizing diverse microbial (bacteria, viruses, fungi and parasites), stress and damage signals, which result in direct activation of caspase-1, leading to secretion of potent pro-inflammatory cytokines and pyroptosis. NLRP3 is the most studied antimicrobial immune response inflammasome. Recent studies reveal expression of inflammasomes in innate immune response cells including monocytes, macrophages, neutrophils, and dendritic cells. Inflammasome deficiency has been linked to alterations in the gastrointestinal microflora. Alterations in the microbiome population and/or changes in gut permeability promote microbial translocation into the portal circulation and thus directly to the liver. Gut derived lipopolysaccharides (LPS) play a significant role in several liver diseases. Recent advancements in the sequencing technologies along with improved methods in metagenomics and bioinformatics have provided effective tools for investigating the 10^{14} microorganisms of the human microbiome that inhabit the human gut. In this review, we examine the significance of inflammasomes in relation to the gut microflora and liver. This review also highlights the emerging functions of human microbiota in health and liver diseases. (J Clin Exp Hepatol 2013;3:141–149)

Inflammasomes

Inflammation is the defense mechanism of the body following tissue injury. In health, this defense mechanism is smoothly switched on and off following initiation and cessation of injury. However, continuous exposure to the noxious stimulus leads to chronic inflammation, parenchymal cell loss, healing by fibrosis and, in the case of the liver, eventually to liver cirrhosis. This is the common pathogenetic process underpinning many chronic liver diseases. The process of inflammation involves innate immune cells and production of pro-inflammatory cytokines IL-1α, IL-1β, and TNF-α.[1]

Macrophages are important cells of the innate immune response and play a crucial role in the initiation and resolution of inflammation. They perform three key functions, namely phagocytosis and destruction of infectious agents, antigen presentation and immune modulation, and initiating the release of various cytokines and growth factors.[2–4] Two types of macrophages have been described. M1 macrophages or immune effector cells engulf and digest damaged cells. They are activated by pro-inflammatory mediators such as lipopolysaccharides (LPS), IL-1β and IFN-γ. In turn, these cytokines produce other pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6 and IL-12) which generate reactive oxygen species.[5,6] On the other hand, M2 macrophages function in wound healing and tissue repair and produce the anti-inflammatory cytokine IL-10, which turns off immune system activation.

Disturbances of steady state or acute damage lead to the initiation of inflammation.[7] Several receptors have been studied that distinguish between homeostasis and agents harmful to the host. Some receptors recognize pathogen associated molecular patterns (PAMPs) which are important for the survival of microbes. Thus, pathogens that are devoid of these structures can be explicitly sensed in the tissues.[8] Alteration in tissue homeostasis due to microbial or non-microbial agents causes the release of damage associated molecular patterns (DAMPs). These molecular patterns help in sensing stressed tissue.[9]

INFLAMMASOMES

The term inflammasome was first introduced by Martinon and co-workers.[10] They are large multiprotein complexes which have the ability to sense intracellular danger signals

Keywords: inflammasomes, microbiota, liver disease, inflammation
Received: 22.9.2012; *Accepted:* 29.3.2013; *Available online:* 15.4.2013
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Abbreviations: LPS: lipopolysaccharides; NOD: nucleotide-binding oligomerization domain; NLR: NOD-like receptor; PAMPs: pathogen associated molecular patterns; DAMP: damage associated molecular patterns; AIM2: absent in melanoma 2; IL: interleukin; IFN: interferon; TNF-α: tumor necrosis factor-α; NACHT: domain present in NAIP, CIITA, HET-E (*Podospora anserina* incompatibility, locus protein) and telomerase associated protein; LRR: leucine-rich repeat; MDP: muramyl dipeptide; ATP: adenosine triphosphate; ROS: reactive oxygen species; NAIP: neuronal apoptosis inhibitor protein; HMGB1: high-mobility group box1; BMDMs: bone marrow-derived macrophages; CTB: Cholera toxin B; TLR: toll-like receptor; NK/NKT: natural killer/natural killer T cells; mCMV: mouse cytomegalovirus; NAFLD: non-alcoholic fatty liver disease; CARD: caspase activation and recruitment domain
http://dx.doi.org/10.1016/j.jceh.2013.03.225

through NOD-like receptors or NLRs.[11] NOD-like receptors are members of the pattern recognition receptor family. Two domains that play a key role in the activation of the inflammasome are the C-terminal leucine rich repeat (LRR) domain and the N-terminal domain present in NAIP, CIITA, HET-E (*Podospora anserine* incompatibility locus protein) and telomerase associated protein (NACHT) domain. Recognition of the ligand depends on the leucine rich-repeat (LRR) domain whereas oligomerization and dNTPase activity are functions of the central NACHT domain.[6] A complex is formed between the NLR sensor and the effector molecule, pro-caspase-1, which may or may not require an adaptor molecule such as apoptosis-associated speck-like caspase activation and recruitment domain (CARD) domain containing protein (ASC).[10–12] Activation of the inflammasome is a two-step process. Signal 1, due to toll-like receptor (TLR) activation, helps in up-regulating expression of the inflammasome while signal 2 triggers its functional activation with the help of inflammasome ligand. Although, many inflammasomes have been discovered till date, such as: NLRP1, 2, 3, 6, 10, 12, NLRC4 and AIM2, we will elaborate the main inflammasome prototypes in this review namely: NLRP1 (NALP1), NLRP3 (NALP3, cryopyrin), NLRC4 (IPAF) and AIM2.[11,12] Although differing in ligand recognition sites and utilization of adaptor molecules, the core function of different inflammasomes is activation of caspase-1.

NLRP1 Inflammasome

The first inflammasome described consists of NACHT, PYD (pyrin domain) and LRR domains. The C-terminal CARD domain interacts directly with caspase-1. Importantly, activity of NLRP1 inflammasome is further enhanced by the presence of ASC.[13] Activation of NLRP1 is triggered by (muramyl dipeptide) and the lethal *Bacillus anthracis* toxin.[14–17] A unique feature of NLRP1 is that it can localize in the nucleus unlike other inflammasomes which are distributed in the cytoplasm.[18]

NLRP3 Inflammasome

Hoffman and co-workers (2001) first described the NLRP3, the most elaborately characterized inflammasome, containing NACHT, LRR and PYD domains containing protein 3 or cryoporin.[19]. Absence of CARD domain makes it important for the ASC molecule to be present for complex formation.[13] Three main pathways have been described for activation of this inflammasome. The first is potassium efflux, due to the presence of P2X7 purinergic receptors that sense extracellular ATP, which recruits pannexin and causes activation of NLRP3.[20–22] Secondly, crystals or large particles such as silica, asbestos, aluminum, amyloid, monosodium urate and cholesterol also lead to activation of NLRP3 inflammasome.[23–31] Thirdly, activation of NLRP3 inflammasome also depends on reactive oxygen species (ROS), as suggested by a study in which blocking of priming by ROS inhibitors prevented activation of the NLRP3 inflammasome.[12]

NLRC4 Inflammasome

NLRC4 inflammasome contains protein 4 and is activated by flagellin of Gram-negative and Gram-positive bacteria as well as type III secretion system (T3SS) of Gram-negative bacteria.[32–34] However, the mechanism involving activation of this inflammasome is not yet fully understood. Although other NLR proteins, such as murine 143 NAIP5 and NAIP2 also interact with bacterial flagellin or type III secretion system (T3SS), it is the rod components that cause the assembly and activation of NLRC4 inflammasome.[35] Recently, it has been shown that *Salmonella typhimurium* infection of NLRP4 macrophages causes phosphorylation of NLRC4 S533. This is followed by the conformational changes necessary for NLRC4 inflammasome activity and host innate immunity.[36]

AIM2 Inflammasome

AIM2 is a cytosolic inflammasome which senses dsDNA. It is activated by bacterial, viral and mammalian host DNA, causing caspase-1 activation.[37–39] AIM2 can bind directly to its ligand. Strikingly, it recognizes the mammalian DNA which acts as a contributory factor toward the pathogenesis of autoimmune diseases.[40] The association of helicase receptor RIG of dsRNA with the inflammasome adaptor molecule ASC leads to inflammasome activation which triggers caspase-1 activation.[41]

Inflammasome Activates Caspase-1

Inflammatory processes involve pro-inflammatory cytokine IL-1β.[42] Though synthesis of IL-1β does not require any signal sequence, interestingly, its activation and release from cellular compartment is dependent on the cysteine protease, caspase-1. Following the protein secretion pathway, caspase-1 also contributes to the processing and secretion of IL-18.

Moreover, IL-1α and fibroblast growth factor-2 are also secreted by the caspase-1 mechanism.[43] Bergsbaken and group studied the role of caspase-1 in pyroptosis, which is programmed cell death involving both apoptosis (DNA fragmentation) and necrosis (inflammation and releasing cytokines).[44] A recent study supported that pyroptosis occurs due to altered secretion and release of IL-1β.[45] Basically, caspase-1 becomes proteolytically active by controlled dimerization in the inflammasome where it is synthesized as the inactive zymogen, pro-caspase-1. Figure 1 depicts the different inflammatory caspases in both humans and mice. Caspase-1 activating complex has been called the inflammasome.[10] The sequence of