Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome and is the most common cause of chronic liver disease in the developed countries, comprising 20–30% of the population.\(^1\)–\(^4\) In developing countries the disease is also the commonest liver ailment.\(^3\) The spectrum of NAFLD ranges from simple steatosis to cirrhosis and hepatocellular carcinoma (HCC); non-alcoholic steatohepatitis (NASH, prevalence 3–6%) may be considered as an intermediate stage of liver damage that has higher chances of progressing to advanced disease, while simple hepatic steatosis progresses very slowly.\(^1,2\) Projections for 10 years from now indicate that that NASH-related cirrhosis would be the leading cause of liver transplantation in the United States.\(^2,5\)

The pathogenesis of this disorder is incompletely understood and no treatment has proven to be effective. A ‘two hit’ theory has been proposed to explain pathogenesis of NAFLD/NASH. The ‘first hit’, hepatic steatosis, involves accumulation of lipids in the form of triglycerides.\(^2\) This lipid-rich environment then provides the optimum setting for additional pro-inflammatory insults including increased lipid peroxidation and reactive oxygen species (ROS) generation, the ‘second hits’, that triggers hepatocellular injury, inflammation, and fibrosis. Flavell and colleagues reported in a recent issue of Nature that dysbiosis driven by defective inflammasome may govern the rate of progression of multiple metabolic syndrome-associated abnormalities, including the rate of NAFLD progression.\(^6\) This study highlights the central role of the microbiota in the pathogenesis of NAFLD.

Inflammasomes are cytoplasmic multi-protein complexes composed of one of several NOD-like receptor (NLR) and the PYHIN (pyrin and HIN200 [hematopoietic interferon-inducible nuclear antigens with 200 amino-acid repeats] domain-containing protein) proteins. There are four main prototypes of inflammasomes, which include NLRP1, NLRP3, NLRC4, and absent in melanoma 2 (AIM2).\(^6,7\) They are sensors of endogenous or exogenous pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), which govern cleavage of effector pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18. Caspase-1 is responsible for the processing and secretion of IL-1β and IL-18. AIM2 and NLRC4 inflammasomes are activated by specific PAMPs, double-stranded DNA (dsDNA) and specific bacterial proteins while NLRP3 is activated by a large variety of signals, including PAMPs, DAMPs, and bacterial toxins.\(^7\) Most DAMPs can activate the NLRP3 inflammasome via the generation of ROS. Flavell and colleagues proposed that inflammasome-dependent processing of IL-1β and IL-18 could play an important role in the progression of NAFLD\(^6\) and have conclusively demonstrated that the NLRP6 and NLRP3 inflammasomes and the effector protein IL-18 negatively regulate NAFLD/NASH progression via modulation of the gut microbiota. The main findings of this study may be summarized as furnished below.

**Role of Inflammasomes in Non-alcoholic Steatohepatitis Progression**

The authors demonstrated more severe liver disease (significantly higher serum alanine aminotransferase and aspartate aminotransferase activity, enhanced microvesicular and macrovesicular hepatic steatosis, and accumulation of multiple immune subsets in the liver from the innate and adaptive arms of the immune system) in C57Bl/6 wild-type (NCI), apoptosis-associated speck-like protein containing a CARD (Asc\(^−/−\), also known as PyCARD) and caspase 1 (Casp1\(^−/−\)) mutant mice compared to wild-type mice on methionine-choline-deficient diet (MCDD).

**Increased Non-alcoholic Steatohepatitis in Asc- and Casp1-deficient Mice was Mediated by IL-1β or IL-18**

Increased severity of NASH was observed in IL-18-deficient (Il18\(^−/−\)) mice, but not IL-1 receptor-deficient (Il1r\(^−/−\)) mice, when fed the MCDD. These observations confirm a key role for IL-18 in negative regulation of disease progression.

**Role of the NLRP3 Inflammasome in Non-alcoholic Steatohepatitis Progression**

Nlrp3\(^−/−\) mice developed exacerbated NASH compared to wild-type mice, suggesting its role in negative regulation of disease progression.

**Enhanced Non-alcoholic Steatohepatitis Severity in Inflammasome-deficient Mice is Driven by Their Altered Microbiota**

The authors have recently demonstrated that a deficiency in components of NLRP6 and NLRP3, both of which include ASC and caspase 1, and involve IL-18 but not IL-1R, results in the development of an altered transmissible,
NAFLD PROGRESSION

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REFERENCES


Toll-like Receptors Signaling Mediates the Increased Susceptibility to Progression to Non-alcoholic Steatohepatitis

Toll-like receptors (TLR) play a major role in pathogenesis of NAFLD because liver is exposed to large amounts of PAMPs derived from the intestine via the portal circulation. Mice without TLR signaling capability (Myd88<sup>-/-</sup>; Trif<sup>-/-</sup>) did not show increased NASH after being co-housed with Asc<sup>-/-</sup> mice and fed on MCDD, indicating TLR signaling (TLR4 and TLR9 activation) mediates the increased susceptibility to progression to NASH. The levels of TLR4 and TLR9 agonists, but not TLR2 agonists, were markedly increased in the portal circulation of MCDD-fed inflammasome-deficient mice or their co-housed partners compared to wild-type controls.

Downstream Mechanism of Microbiota-induced Toll-like Receptors Signaling

Tumor necrosis factor (TNF)-α is a downstream cytokine of TLR signaling and is a pro-inflammatory cytokine; it is responsible for progression of hepatic steatosis to steatohepatitis. Hepatic TnfmRNA expression was significantly up-regulated in Asc<sup>-/-</sup> and Il18<sup>-/-</sup> mice following induction of NASH by MCDD; Enhanced expression of TNF-α was mediated by elements of the microbiota responsible for NASH exacerbation.

This landmark study provide direct evidence that modulation of the intestinal microbiota through multiple inflammasome components is a critical determinant of NAFLD/NASH progression. NLRP3- and NLRP6-inflammasome-deficient (IL-18 deficiency) mice develop alterations in gut microbiota (abundance of Prevotellaceae and Porphyromonadaceae species), which results in the translocation of bacterial products derived from the intestine into the portal circulation. These bacterial products trigger TLR4 and TLR9 activation and subsequently TNF-α, a downstream pro-inflammatory cytokine, mediated hepatotoxicity that results in an increased rate of NAFLD progression.

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