Role of pattern recognition receptors (PRR) in the pathogenesis of non-alcoholic steatohepatitis (NASH)

Doctoral thesis

Tímea Csák M.D.

Semmelweis University
Clinical Medicine School of Doctoral Studies

Supervisor: Gyöngyi Szabó M.D., PhD

Reviewers: Gabriella Pár M.D., PhD.
Klára Werling M.D., PhD.

Comprehensive exam committee:
President: János Banai M.D., CSc
Members: László Herszényi M.D., PhD.
György Székely M.D., CSc

Budapest
2012

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases affecting over 1/3 of the population in the Western world. The histopathology spectrum of NAFLD includes steatosis alone, steatosis with inflammation and steatohepatitis (non alcoholic steatohepatitis/NASH) that includes necroinflammation with or without fibrosis. (1). The pathogenesis of NAFLD/NASH is not fully understood yet. Recently the role of innate immunity has been implicated in the pathogenesis of NASH (1). Pattern recognition Toll-like receptors (TLR) and NOD-like receptors (NLR) are key components of the innate immune system in the recognition of pathogens, but they also sense danger signals released from damaged cells (94). The inflammation induced via the TLRs or NLRs contribute to the pathogenesis of several autoinflammatory diseases.

Fatty liver is highly sensitive to the TLR4 ligand lipopolysaccharide (LPS or endotoxin), that is a bacterial wall component of Gram-negative bacteria. Furthermore, increased plasma endotoxin levels were detected in steatohepatitis both in mice and humans. However, the role of the TLR4-MD2 receptor complex in NASH is yet to be evaluated.

Inflammasomes, large caspase-1-activating multiprotein complexes that sense both danger signals through the intracellular NLRs, are major contributors to inflammation. The NALP3 inflammasome is involved in sensing endogenous danger signals, and promotes the cleavage and maturation of the pro-inflammatory cytokine pro-IL-1β to promote/sustain inflammation. The inflammasome activation is a two step process, in which the first step usually by a TLR ligand such as LPS induce the up-regulation of the inflammasome and pro-IL-1β, and a second signal activate the inflammasome. The cell-specific expression and role of the inflammasome in the liver are yet to be evaluated in NASH.

While the factors determining progression of NASH are yet to be fully defined, the clinical importance of increased susceptibility of the fatty liver to viral infections is emerging. Co-morbidity of NASH with viral infections caused by RNA viruses, such as hepatitis C and HIV remains a clinical challenge. The pathomechanism behind the impaired antiviral immuntity is not fully clarified yet.

2. AIMS

The aim of thesis was to explore the role of innate immunity in the pathogenesis of NASH.

2/1. The first part of the work focus on the role of the Gram negative bacterial wall component endotoxin and its receptor, Toll-like receptor 4 in the development of diet-induced steatohepatitis and fibrosis. To perform the experiments we employed wild type, TLR4- and MD2 (myeloid differentiation factor 2)-deficient mice fed with methinone-choline deficient (MCD) diet to induce steatohepatitis. MD2 is the part of the LPS-sensing TLR4 receptor complex.

2/2. The second part of the work was designed to investigate the role of the pro-inflammatory cytokine IL-1β and the inflammasome complexes that are responsible for the IL-1β-maturation in the pathogenesis of NASH. We wanted to answer the following questions:

- Is there inflammasome activation and increased IL-1β production in animal models of liver steatosis and steatohepatitis?
- Which cell type(s) is/are involved in the inflammasome activation in NASH? Do hepatocytes express the inflammasomes?
- What does activate the inflammasome in NASH?
- Does the inflammasome activation contribute to the development / progression of NASH? To perform the experiments we employed the MCD diet model of steatohepatitis on wild type, ASC, caspase-1 and IL-1R deficient mice.
- Is there increased inflammasome expression in liver biopsy samples of NASH patients?

2/3. In the third part of the work we aimed to explore the pathogenesis behind the susceptibility of fatty liver to viral diseases. We tested the hypothesis that mice with steatohepatitis are more susceptible to virus induced liver injury. To perform the experiment we employed mice fed with MCD diet to induce steatohepatitis and challenged them with Poly I:C to mimic viral infection.

Arra kerestük a választ, hogy vajon azok az egerek, amelyekben NASH-t hoztunk létre methionin és cholin hiányos diétával, fogékonyabbak-e a vírus indukálta májkárosodásra. Továbbá vizsgálatunk célja volt a kiváltott antivirális válasz részletes tanulmányozása. We investigated the intracellular signaling cascade step by step induced by Poly I:C.

METHODS

Animal studies

This study was approved by Institutional Animal Use and Care Committee (IACUC) at University of Massachusetts (UMASS) Medical School.

Wild type (wt) C57Bl/6 mice were fed with either methionine-choline deficient (MCD) diet for 5 or 8 weeks; or high fat diet (HFD) for 4 weeks or 9 months. Control mice received either an MCD-identical, but DL-methionine and choline bitartrate supplemented (MCS) diet, or regular rodent chow diet. We also used 9 weeks old, female leptin deficient (ob/ob; B6.V-Lep ob/J) mice with their own age and gender-matched control group. TLR4 ligand lipopolysaccharide (LPS) or TLR3/RLR ligand polyinosinic:polycytidylic acid (Poly I:C), a synthetic double stranded RNA; or TLR9 ligand CpG-ODN were injected intaperitoneally for 2 or 6 hours. The following knock-out mice were used: TLR4-, MD-2, ASC-, caspase-1or IL-1R-defcient mice with their appropriate controls.

We performed *biochemical analysis and cytokine measurements* (serum alanine aminotransferase, serum cytokines [TNFα, IL-6, IL-1β, IFNβ, HMGB1], liver triglyceride and thiobarbituric reactive substances [TBARS]; *histopathological analysis* (hematoxylin-eosin, picro-sirius red, OilRed O staining, F4/80 and α–smooths muscle actin immunohistochemistry). RNA and protein (whole cell, mitochondrial and cytoplasmic) were isolated from liver tissue and used for real time PCR and Western blot (SDS-PAGE and native gel electrophoresis, immunoprecipitation) analysis, respectively. The following functional assays were performed: caspase-1, -3, NADPH activity and cell cytotoxicity assays.

In vitro experiments

Hepatocytes and liver mononuclear cells were isolated from mice, and we also employed mouse hepatoma (Hepa1-6) and monocyte-macrophage (RAW 264.7) cell lines.The cells were stimulated with LPS, saturated and non-saturated fatty acids (palmitic, oleic, linoleic acid) or their combinations with or without pan-caspase inhibitor. Poly I:C was used to stimulate hepatocytes with or without Lipofectamin 2000. Liver mononuclear cells were analysed by flow cytometry for TNFα and ED1 (CD68) positivity.

Human liver samples

The study meets the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Committee for the Protection of Human Subjects in Research at the University of Massachusetts. All participants gave a written consented to participate in the study. Human liver tissue was obtained from biopsies from six, clinically and biopsy-proven NASH patients. Human liver tissue from chronic hepatitis C infected patients (n=5) were used as diseased controls. Human normal liver (n=4) total RNA was purchased from *OriGene Technologies.*

RESULTS

Deficiency in myeloid differentiation factor-2 (MD2) and toll-like receptor 4 (TLR4) expression attenuates non-alcoholic steatohepatitis and fibrosis in mice

MD-2 or TLR4 protects from MCD diet-induced liver fat deposition and inflammation

MD-2 and TLR4 complex is the major receptor for endotoxin that has been shown to contribute to activation of the inflammatory cascade in alcoholic steatohepatitis (ASH) leading to liver damage. Given the common pathophysiological features of ASH and NASH, we aimed to identify the role of MD-2/ TLR4 complex in an experimental model of NASH.

Mice of control genotypes fed a methionine-choline-deficient (MCD) diet for 8 weeks developed significant hepatic steatosis and inflammation. MD-2- and TLR4-deficient mice on MCD diet showed lower liver fat accumulation and inflammatory cell infiltration compared to the mice of control genotypes. Latter one was showed by the reduced number of F4/80+ and CD68+ TNFα-producing macrophages, as well as by the lower serum TNFα levels in the knock out mice compared to the mice of control genotypes. A significant increase in serum alanine aminotransferase (ALT), suggesting on-going liver damage, was observed in the MCD-diet-fed control genotype mice, however, the ALT increase was significantly attenuated in MD-2- and TLR4-deficient mice.

MD-2 and TLR4 deficiency attenuates oxidative stress

Increased lipid peroxidation and oxidative stress are keys in development of non alcoholic steatohepatitis. We identified significantly higher levels of liver thiobarbituric acid substances (TBARS), indicative of lipid peroxidation, in MCD-diet compared to the MCS diet-fed genotype control mice. Consistent with our hypothesis that MD2/TLR4 complex plays a role in NASH, we found significantly reduced induction of TBARS in the livers of MCD-diet-fed MD-2- and TLR4-deficient mice. NADPH oxidases play an important role in the generation of reactive oxygen radicals.

NADPH oxidase expression revealed a significant upregulation and activation of the NADPH oxidase complex (p47phox, p67phox, gp91phox, p22phox) in MCD-diet-fed animals of control genotypes. Deficiency in MD-2 or TLR4 abrogated the MCD-induced up-regulation of all of the NADPH oxidase subunits, suggesting that NADPH-mediated oxidative stress is dependent on MD-2 and TLR4 expression in this model.

MD-2 and TLR4 deficiency protects from NASH-associated liver fibrosis

A key clinical challenge in human NASH is its progression to fibrosis and cirrhosis. MCD diet feeding for 8 weeks results in hepatic stellate cell (HSC) activation and fibrosis in mice, that was showed by the αSMA immunohistochemistry, sirius red staining and the increased expression of the fibrosis-related genes (αSMA, collagen, TGFβ). We found that the deficiency of either MD-2 or TLR4 attenuated significantly

the MCD-diet induced fibrosis compared to the mice of control genotypes. Liver fibrosis involves inflammation-driven tissue remodeling; matrix metalloproteinases (MMP) and their specific tissue inhibitors (TIMPs) closely regulate the metabolism of the extracellular matrix. Bothe the MMP-2 and TIMP-1 expression were increased in MCD diet-fed mice compared to MCS-diet-fed mice of control genotypes; the induction of these genes was significantly attenuated in the absence of MD-2 or TLR4 expression.

Fatty acids and endotoxin activates the inflammasome in non-alcoholic steatohepatitis

Given the role of endotoxin and TLR4, and the fact that there is crosstalk between TLR and NLR signaling, furthermore, that endotoxin is a known activator of inflammasomes, next step we aimed to investigate the role of inflammasomes in the pathogenesis of NASH. Inflammasomes are large, intracellular multiprotein complexes that sense intracellular danger signals via NOD-like receptors and lead to caspase-1 activation and IL-1β secretion.

<u>MCD diet-induced steatohepatitis is associated with increased IL-1β production and inflammasome activation in the liver</u>

The MCD diet model of non-alcoholic steatohepatitis (NASH) is characterized by steatosis and prominent inflammation. Here we found that among other pro-inflammatory cytokines the levels of serum IL-1β as well as hepatic IL-1β mRNA and protein were significantly increased in the livers of MCD diet-fed mice compared to MCS controls. Consistent with this we found increased expression and activation of the NALP3 inflammasome complex (NALP3, ASC, pro-caspase-1) in the livers of MCD diet-fed mice compared to MCS-controls. NALP3 forms complex with pro-caspase-1 via the adaptor ASC and after the auto-activation of the caspase-1 enzyme, the active caspase-1 cleaves and activates pro-IL-1β. The increased caspase-1 activity was accompanied by higher cleaved, mature IL-1β protein levels in MCD-steatohepatitis compared to the controls.

Long-term, but not short-term high fat diet feeding is associated with inflammasome activation in the liver

While the MCD diet model induces NASH, high fat diet results in steatosis after 4 weeks and evidence of inflammation occurs after prolonged HFD feeding. Consistent with this, we observed an increase in liver TNFα-expression only in livers with 9-month and not with 4-week HFD feeding. We found that 4-week HFD resulted in no increase in inflammasome expression, while 9-month HFD induced significant up-regulation of the NALP3 inflammasome complex at the mRNA level. Inflammasome activation was indicated by increased caspase-1 activity and higher liver mature IL-1β protein levels in 9-month but not in 4-week HFD groups compared to their corresponding controls.

Liver steatosis without features of inflammation is also prominent in leptin deficient (ob/ob) mice. We found no inflammasome activation in ob/ob mice compared to their controls.

Increased inflammasome expression in human NASH

There was a significant increase in inflammasome gene expression including NALP3, pro-caspase-1, ASC and pannexin-1 in livers from NASH patients compared to healthy controls. Liver samples from chronic HCV infected patients also showed increased inflammasome expression, however, to a lower extent than NASH livers.

LPS induces upregulation of the inflammasome in the liver

In vivo stimulation with LPS, lead to up-regulation of the hepatic inflammasome components at the mRNA level and increased IL-1β protein in the liver in both MCD and MCS diet-fed mice. However, the LPS-induced inflammasome expression was higher in MCD diet-fed mice compared to MCS controls.

Inflammasome is upregulated in hepatocytes in NASH

Next step, we sought to evaluate whether inflammasome activation occurs in hepatocytes. To date inflammasome expression and activation has been mostly studied in innate immune cells. We found that primary hepatocytes of MCD diet-fed mice showed increased expression of NALP3, ASC, pro-caspase-1, pannexin-1 and pro-IL-1beta mRNA compared to controls, but not the liver mononuclear cells.

Fatty acids and LPS induce inflammasome activation in hepatocytes

Both circulating fatty acids and gut-derived endotoxins (LPS) contribute to the pathogenesis of NASH. We found increased serum endotoxin levels in mice with steatohepatitis. Palmitic acid, a saturated fatty acid induced increased expression of NALP3 mRNA in isolated mouse hepatocytes, as well as Hepa 1-6 cells and RAW macrophages. LPS also induced NALP3 mRNA expression in the hepatocytes. However, LPS alone did not result in inflammasome activation and IL-1β secretion, and palmitic acid also induced only moderate increase in IL-1ß protein secretion without detectable caspase-1 activation. Significantly higher levels and earlier IL-1ß production was seen in hepatocytes with palmitic acid pre-treatment followed by LPS stimulation compared to PA or LPS treatment alone, suggesting sensitization in hepatocytes.

The observation that palmitic acid alone induced IL-1ß secretion without extensive evidence of caspase-1 activation prompted us to evaluate alternative mechanisms for IL-1 cleavage in hepatocytes. While pro-IL-1β cleavage is mostly a result of inflammasome-mediated caspase-1 activation, it can also be cleaved by caspase-8. Indeed, we found that palmitic acid, but not LPS, resulted in caspase-8 activation and more importantly, caspase-8 activation was not increased by the combination of palmitic acid and LPS. These results suggested that caspase-8 could be involved in the IL-1β cleavage in PA-treated hepatocytes.

Palmitic acid-treated hepatocytes transmit danger signals and induce inflammasome activation in liver mononuclear cells

Caspase-8 is also induced in apoptosis. Increased LDH release in hepatocytes after PA treatment indicated induction of cell death. We determined that up-regulation of NALP3 and IL-1β mRNA by palmitic acid was caspase-dependent because these events were prevented by addition of the pan-caspase inhibitor, ZVAD in hepatocytes. This observation also suggested that damage-associated molecules generated in apoptotic hepatocytes rather than palmitic acid itself could contribute to inflammasome activation. Furthermore, these danger molecules can activate the inflammasome in the liver mononuclear cells. PA-free supernatants from PA-pretreated hepatocytes induced upregulation of NALP3 and IL-1β mRNA in the LMNCs in a ZVAD-dependent manner.

Deficiency of the inflammasome components does not prevent liver injury and fat deposition in the MCD-diet model of NASH

Next step we tested the physiological significance of inflammasome activation in NASH using various knock-out mice. Neither the deficiency of ASC (inflammasome adaptor), nor the deficiency of caspase-1 (inflammasome effector) prevented the MCD diet-induced liver injury, indicated by the high ALT levels both in the wild type and knock out genotypes. ASC-deficiency did not influence the development of steatosis and we detected compareable IL-1β levels in the liver of bothe the knock out and the control genotypes. This suggested that inflammasomes that do not need ASC as an adaptor molecule (eg. NALP1) might also contribute to the pathogenesis of NASH. However, the deficiency of caspase-1 did not influence either the active IL-1β levels in the liver suggesting the possibility of alternative IL-1β cleavage. As we mentioned above, among others, caspase-8 is also able to cleave IL-1β. Consistent with this we found increased caspase-8 activity in MCD diet-induced steatohepatitis in wild type, ASC- and caspase-1-deficient mice. This suggested that caspase-8 might compensate the caspase-1 deficiency in terms of IL-1β cleavage in NASH.

Interleukin-1 receptor deficiency attenuates hepatic steatosis, but does not prevent MCD-diet-induced liver injury or fibrosis

To further explore the role of IL-1β in NASH, we employed IL-1 receptor (IL-1R) knock out mice. We found attenuated steatosis in MCD-diet fed IL-1R KO mice compared to WT controls. In contrast, IL-1R deficiency failed to prevent liver injury and fibrosis in MCD-diet-fed mice Latter one was shown by the collagene mRNA levels, sirius red staining and αSMA immunohistochemistry.

Mitochondrial antiviral signaling protein defect links impaired antiviral response and liver injury in steatohepatitis in mice

Type-I IFN induction is decreased in steatohepatitis in response to poly I:C stimulation

Polyinosinic-polycytidylic acid (poly I:C), a synthetic double-stranded RNA (dsRNA), is a surrogate for viral infection. Double stranded RNA is recognized by TLR3 and helicase receptors and induces robust Type-I IFN response leading to anti-viral immunity. We found significantly decreased poly I:C-induced Type-I interferon production in mice with MCD-steatohepatitis compared to MCS controls. There were significantly lower serum IFNβ, liver IFNβ and –α4 protein and mRNA levels, as well as hepatic mRNA levels of Type-I IFN inducible genes (ISG56, ISG15) in MCD-steatohepatitis compared to MCS controls after poly I:C stimulation.

Impaired Type-I IFN induction in steatohepatitis is restricted to the RIG-I/Mda5 pathway

To further evaluate the significance of impaired Type-I IFN induction in steatohepatitis, we employed stimulations that induce Type-I IFNs via receptor pathways different from dsRNA recognition by TLR3 and its adapter, TRIF, or RIG-I/Mda5 and their adapter MAVS, respectively. LPS is recognized by TLR4 and uses the adapters TRIF and MyD88, while CpG DNA, a ligand for TLR9 solely utilizes the MyD88 adapter in Type-I IFN induction. The fact that impaired Type-I IFN production in steatohepatitis was observed only in case of dsRNA stimulation suggested a selective impairement of the helicase receptor signaling and not of the TLR3/TRIF-dependent pathways.

Abnormal MAVS function in NASH involves decreased protein levels, dissociation from the mitochondria and impaired oligomerization

The adapter molecule MAVS is critical for the downstream signaling of helicase receptors and its dysfunction impairs proinflammatory cytokine and interferon induction via the NFκB and IRF3 signaling pathways, respectively. Consistent with decreased induction of Type-I IFN, we found decreased levels of MAVS protein in whole liver lysates of MCD-diet fed mice compared to controls. In search of possible mechanisms for decreased MAVS protein levels despite of the increased MAVS mRNA expression, we found higher mRNA expression of the PSMA7 subunit of proteasome in MCD steatohepatitis. PSMA7 can negatively regulate MAVS-mediated immune responses and promotes proteosomal degradation. Immunoprecipitation experiments revealed increased association between MAVS and PSMA7 in fatty livers compared to controls.

We also found that steatohepatitis resulted in decreased mitochondria-associated MAVS protein levels and decreased MAVS oligomerization compared to controls. Both the mitochondrial localization and oligoemrization of MAVS is crucial for Mda5/RIG-I activation. Consistent with this, we found impaired poly I:C-induced IRF3 phosphorilation in MCD-steatohepatitis compared to the controls.

Mitochondrial damage occurs in the fatty liver

Mitochondrial dysfunction plays a role in the pathogenesis of NASH and upon mitochondrial damage, its content leaks into the cytosol triggering diverse signaling pathways, including apoptosis. Indeed, mitochondrial damage was indicated by relocation of cytochrome C from the mitochondria to the in MCD-steatohepatitis compared to the controls. Mitochondrial damage in NASH has been linked to excessive levels of reactive oxygen species (ROS). We detected significantly increased liver TBARS levels indicating ROS-induced lipid peroxidation at baseline and after poly I:C stimulation in steatohepatitis. Mitochondrial damage might contribute to the abnormal MAVS localization and function. In addition, we found increased caspase-8 and -1 activity in MCD-steatohepatitis. Both enzymes has been shown to cleave and inactivate MAVS protein.

Increased poly I:C-induced liver damage occurs without excessive pro-inflammatory cytokine induction in steatohepatitis

Poly I:C challenge significantly increased liver injury in MCD diet-fed mice indicated by significantly increased serum ALT levels compared to MCS controls mice. Because dsRNA-induced activation of helicase receptors leads to Type-I IFN induction as well as activation of NFκB and production of pro-inflammatory cytokines, we sought to evaluate whether the increased liver damage was the consequence of enhanced pro-inflammatory cytokine production in steatohepatitis. At baseline, MCD diet-fed mice showed increased serum and liver mRNA levels of TNFα, IL-6 and IL-1β compared to MCS controls. While poly I:C challenge increased TNFα, IL-6 and IL-1β production both in controls and MCD-diet fed groups, the extent of pro-inflammatory cytokine protein and mRNA induction was significantly lower in MCD compared to MCS diet-fed mice. These data suggested that it is less likely that the pro-inflammatory cytokines

account for the increased liver damage in NASH. Since, previous studies showed a crucial role for NK cells in poly I:C induced liver injury, we next investigated the possible role of NK cells. We found increased mRNA expression of the NK-activating ligands (Pan-Rae, Rae-1α, Mult-1) in MCD-steatohepatitis, but poly I:C did not induce a further increase in the expression of these ligands.

Poly I:C promotes a switch from apoptosis to necrosis and increases RIP3 expression in steatohepatitis

Hepatocyte apoptosis is a key component of liver damage in NASH. Consistent with this we found higher caspase-3 activity in MCD diet-fed mice compared to the controls. Poly I:C induced apoptosis in the livers of MCS diet-fed control mice indicated by the higher caspase-3 activity, but it did not increase further the apoptosis in MCD-steatohepatitis. In contrast, the poly I:C-induced serum HMGB1 levels were significantly higher in the MCD diet-fed mice comapred to MCS controls suggesting that the presence of necrosis. A recently identified master regulator between apoptosis and necrosis is the protein kinase receptor-interacting protein 3 (RIP3). We found increased levels of RIP3 mRNA and protein in livers of MCD- compared to MCS-diet-fed controls. In control mice poly I:C stimulation induced upregulation of RIP3 protein expression at 2 hours post-stimulation which returned to baseline by 6 hours; in contrast, there was sustained induction of RIP3 in steatohepatitis after poly I:C challenge. We further identified a positive correlation between RIP3 and liver HMGB1 expression. Collectively, these data suggested that pathways that promote necrosis are preferentially upregulated in steatohepatitis after a viral challenge, at least in part due to the regulatory involvement of RIP3.

Altered MAVS and RIP3 mRNA expression in human NASH

Consistent with our observations in the animal models, we found higher MAVS, PSMA7 and RIP3 mRNA levels in human NASH livers comapred to healthy controls and compared the hepatitis C infected patients.

CONCLUSIONS, SUMMARY

In the present study we demonstrate several novel findings that support the substantial role of innate immunity in the pathogenesis of NASH.

The following conclusions can be drawn based on our results:

1. Deficient integrity of the danger receptor complex, including TLR4 or its co-receptor MD-2, is protective from MCD-diet-induced liver steatosis, inflammation, correlates with attenuated liver injury and confers protection from development of liver fibrosis in MCD-diet-induced NASH.

2. There is up-regulation and activation of the NALP3 inflammasome complex and therefore increased IL-1β production in NASH in mouse models as well as in human livers. Our data also suggest that inflammasome activation occurs in steatohepatitis and not in early steatosis in mice.

3. While increased circulating endotoxin likely contributes to inflammasome activation, exogenous LPS can amplify inflammasome activation and IL-1ß secretion in steatohepatitis.

4. Inflammasome activation and IL-1ß secretion occur in isolated hepatocytes in NASH.

5. Saturated, but not un-saturated, fatty acids increase inflammasome expression and sensitize hepatocytes to IL-1ß release by a second stimulus via TLR4 activation.

6. Fatty acids not only upregulate inflammasome but also induce apoptosis and release of danger signals in hepatocytes.

7. These danger signals induce inflammasome activation in liver mononuclear cells demonstrating a cross-talk between injured hepatocytes and inflammatory cells in NASH.

8. The IL-1 signaling contributes to the development of liver steatosis; however, neither the lack of the inflammasome effector caspase-1, nor IL-1R deficiency prevents liver injury and/or fibrosis in MCD diet-induced steatohepatitis.

9. There is increased inflammasome expression in the liver of NASH patients.

Finally, here we report novel findings related to the impaired capacity of the fatty liver to respond to dsRNA and related viral challenges:

10. Livers with steatohepatitis fail to activate anti-viral innate immune pathways to produce Type I IFNs in response to a double-stranded RNA challenge.

11. The MAVS adapter, which is required for Type-I IFN induction after recognition of dsRNA by the helicase receptors RIG-I and Mda5, is dissociated from the mitochondria to the cytosol and shows impaired oligomerization and function in steatohepatitis.

12. The displacement of MAVS from mitochondria is associated with oxidative stress and instead of upregulation of the apoptosis cascade, poly I:C promotes necrosis via increased expression of RIP3 in steatohepatitis.

13. Fourth, we show that dsRNA challenge results in increased liver damage in spite of decreased TNFα and pro-inflammatory cytokine induction in a diet-induced model of NASH.

Our novel data might contribute to better understand the pathogenesis of non alcoholic steatohepatitis and therefore to develop targeted therapy.

PUBLICATIONS

Publications for the dissertation based on:

Csak T#, Velayudham A#, Hritz I, Petrasek J, Levin I, Catalano D, Mandrekar P, Dolganiuc A, Kurt-Jones EA, Szabo G: Deficiency in myeloid differentiation factor-2 and Toll-like receptor 4 expression attenuates non-alcoholic steatohepatitis and fibrosis in mice. (# These authors contributed equally) *Am J Gastroenterol Physiol, Gastrointestinal and Liver Physiology, 2011;300:G433-41.* **IF: 3.522$_{(2010)}$**

Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G: Fatty acids and endotoxin activate inflammasome in hepatocytes which release danger signals to activate immune cells in steatohepatitis. *Hepatology, 2011; 54(1): 133-44.*

IF: 10.885$_{(2010)}$

Csak T, Dolganiuc A, Kodys K, Nath B, Petrsek J, Bala S, Lippai D, Szabo G: Mitochondrial antiviral signaling protein defect links impaired antiviral response and

liver injury in steatohepatitis in mice. *Hepatology, 2011; 53(6):1917-31.*

IF: 10.885$_{(2010)}$

Ganz M, **Csak T**, Nath B, Szabo G: Lipopolysaccharide induces and activates the Nalp3 inflammasome in the liver. World J Gastroenterol 2011; 17(43): 4772-4778.

IF: 2.240$_{(2010)}$

Other publications:

Nagy J, Vincze Z , Folhoffer A, Horváth A, **Csak T**, Zelkó R. A Wilson-kór patomechanismusa és gyógyszeres kezelése. *Acta Pharmaceutica Hungarica* 2003; 73(4): 237-241.

Keresztes K, Istenes I, Folhoffer A, Lakatos PL, Horvath A, **Csak T**, Vargha P, Kempler P, Szalay F: Autonomic and sensory nerve dysfunction in primary biliaris cirrhosis. *World J Gastroenterol* 2004, 10 (20):3039-3043.

Folhoffer A, Horváth A, **Csak T**, Nébenführer L, T, Hazslinszky P, Iványi A, Szalay F. Neurofibromatosis, melanoma malignum, hyperthyreosis és HCV pozitivitás esete. *Lege Artis Medicinae* 2004; 14(5): 349-352.

Szalay F, Telegdy L, Szeli D, **Csák T**, Folhoffer A, Horváth A, Abonyi M, Szabó O, Rédei Cs, Nemesánszky E. Rifaximin a hepaticus encephalopathia kezelésében. *Lege Artis Medicinae* 2004; 14(5): 321-325.

Csák T, Folhoffer A, Horváth A, Lengyel G, Kóbori L, Szalay F: Májtranszplantáció kombinált antivirális kezelése. *Orv Hetil* 2004; 145(39):2003-2006.
Horvath A, Folhoffer A, **Csak T**, Komoly S, Szalay F: Cryoglobulinaemiával és súlyos fokú polyneuropathiával járó krónikus C hepatitis gyógyult esete. *Magyar Belorv Arch* 2004; 57: 194-197.

Nagy J, Folhoffer A, Horvath A, **Csak T**, Taba G, Szentmihályi K, Szalay F, Zelko R:Kinetic study of zinc sulphate release from lipophilic matrices prepared for the therapy of Wilson's disease. *Pharmazie* 2005; 60(7): 524-526. **IF: 0.677**

Szalay F, Folhoffer A, Horvath A, **Csak T**, Speer G, Nagy Zs, Lakatos P, Horvath Cs, Habior A, Tornai I, Lakatos PL: Serum leptin, soluble leptin receptor, free leptin index and bone mineral density in patients with primary biliary cirrhosis. *Eur J Gastroenterol and Hepatol* 2005;17(9):923-928**.** **IF: 1.690**

Keresztes K, Folhoffer A, Lakatos PL, Istenes I, Horváth A, **Csák T**, Vargha P, Kempler P, Szalay F: Az autonom és szenzoros neuropathia gyakorisága és rizikófaktorai primer biliáris cirrhosisban. *Magyar Belorv Arch* 2005; 58; 103-112.

Csak T, Folhoffer A, Horvath A, Halász J, Diczhazi Cs, Schaff Zs, Szalay F: Holmes-Adie syndrome, autoimmune hepatitis and coeliac disease. Case report. *World J Gastroenterol* 2006; 12(9):1485-1487.

Csák T, Folhoffer A, Horváth A, Osztovits J, Halász J, Diczházi Cs, Schaff Zs, Szalay F: Autoimmun hepatitis, coeliakia és Holmes-Adie szindróma együttes előfordulása *Magy Belorv Arch* 2006; 59: 55-58.

Csák T, Folhoffer A, Horváth A, Osztovits J, Papp J, Görög D, Kóbori L, Szalay F: Extrém hypercholesterinaemia és xanthomatosis laparoszkópos cholecystectomia után. Teljes reverzbilitás a iatrogén szűkület műtéti megoldását követően. *Orv Hetil* 2006; 147(15): 705-710.

Horváth A, Folhoffer A, **Csák T**, Osztovits J, Szalay F: Májrák primer biliáris cirrhosisban. Az irodalom áttekintése egy eset kapcsán. *Magyar Belorv Arch* 2006; 61: 207-212.

Folfoffer A, Ferenci P, **Csak T**, Horvath A, Hegedus D, Firneisz G, Osztovits J, Kosa JP, Willheim-Polli C, Szonyi L, Abonyi M, Lakatos PL, Szalay F: Novel mutations of ATP7B gene among 109 Hungarian patients with Wilson disease. *Eur J Gastroenterol Hepatol* 2007, 19(2): 105-111. IF: 1.895

Osztovits J, Horváth T, Abonyi M, Tóth T, Visnyei Z, Bekö G, **Csak T**, Lakatos PL, Littvay L, Fehér J, Kempler P, Kollai M, Szalay F: Chronic hepatitis C virus infection associated with autonomic dysfunction. *Liver Int* 2009; 29(10): 1473-10. **IF: 2.987**

Altorjay I, Vitalis Z, Tornai I, Palatka K, Kacska S, Farkas G, Udvardy M, Harsfalvi D, Dinya T, Orosz P, Lombay B Jr, Par A, Par G, **Csak T**, Osztovits J, Szalay F, Csepregi A, Lakatos PL, Papp M: Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis. *J Hepatol* 2010; 53(3): 484-91. **IF: 9.334**

Papp M, Norman GL, Vitalis Z, Tornai I, Altorjay I, Foldi I, Udvardy M, Shums Z, Dinya T, Orosz P, Lombay B jr, Par G, Par A, Veres G, **Csak T**, Osztovits J, Szalay F, Lakatos PL. Presence of anti-microbial antibodies in liver cirrhosis -- a tell-tale-sign of compromised immunity? *PloS One* 2010; 5(9): e12957 **IF: 4.411**

Bala S, Marcos M, Kodys K, **Csak T**, Catalano D, Mandrekar P, Szabo G: Upregulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor alpha (TNF{alpha}) production via increased mRNA half-life in alcoholic liver disease. *J Biol Chem* 2011;286:1436-44. **IF: 5.328$_{(2010)}$**

Petrasek J, Dolganiuc A, **Csak T**, Kurt-Jones EA, Szabo G: Type-I Interferons protect from Toll-like receptor-9 associated liver injury and regulate IL-1 receptor antagonist in mice. *Gastroenterology* 2011;140:697-708. **IF: 12.032$_{(2010)}$**

Petrasek J, Dolganiuc A, **Csak T**, Nath B, Hritz I, Kodys K, Catalano D, Kurt-Jones EA, Mandrekar P, Szabo G: Interferon regulatory factor 3 and Type I interferons are protective in alcoholic liver injury in mice via cross-talk of parenchymal and myeloid cells . *Hepatology* 2011;53:649-60. **IF: 10.885$_{(2010)}$**

Nath B, Levin I, **Csak T**, Petrasek J, Mueller C, Kodys K, Catalano D, Mandrekar P, Szabo G: Hepatocyte-specific Hypoxia Inducible Factor-1a is a determinant of lipid accumulation and liver injury in alcoholic steatosis in mice. *Hepatology* 2011;53:1526-37. **IF: 10.885 $_{(2010)}$**