Cellular Interactions in the Human Fatty Liver

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Abstract
Non-alcoholic steatohepatitis morbidity and mortality is on the rise due to the obesity pandemic. Its pathophysiology is not well understood and implies complex interactions between local hepatic cell populations, adipocytes, immune effectors that lead to hepatic lipid excess, lipotoxicity, cellular stress and inflammation, as well as programmed cell death. A better understanding of these pathogenic interactions would allow better identification of therapeutic targets in a disease that has no known pharmacological therapy until now.

Keywords: NAFLD, NASH, steatohepatitis, genetics, pathophysiology, natural history

Introduction

Insulin resistance, NAFLD, NASH and cirrhosis

Non-alcoholic steatohepatitis (NASH) became the leading cause of liver transplantation in a world afflicted by obesity epidemics. It is a component of the metabolic syndrome (hypertension, obesity, dyslipidemia, ectopic fat deposits) and, alongside simple steatosis, is one of the faces of non-alcoholic fatty liver disease (NAFLD).

If steatosis is the most widely met manifestation of NAFLD and represents a benign excess of fat within the hepatocyte that seldom leads to complications, NASH implies not only steatosis but also inflammation that in some patients is associated with progressive fibrosis, cirrhosis and occasionally hepatocellular carcinoma. This process seems to be driven by lipotoxicity and cellular metabolism dysfunctions caused by insulin resistance and eventually advances to chronic apoptosis, fibrosis, and cirrhosis. From hepatocytes and immune cells to adipose tissue and endothelium, multiple cellular actors are involved in NASH progression. Their action is modulated by genetic predisposition and environmental factors like diet and intestinal microbiota.

A better understanding of these pathogenic interactions would allow better identification of therapeutic targets in a disease that, until now, has no known pharmacological therapy. This review focuses on how different cells interact and mediate NASH progression.

From fat storage to steatohepatitis

Excess fat, especially triglycerides, stored as droplets within the hepatocytes, seems to be continuously found in both steatosis and NASH. Since triglycerides are probably not hepatotoxic and even protective for the hepatocyte [1], the simple storage excess found in steatosis should not, by itself, explain the progression to NASH and fibrosis and cirrhosis [2]. For example, oleate and other monosaturated and polyunsaturated fatty acids (FAs) are associated with triglyceride-rich droplets production that corresponds to simple steatosis and are considered protective as to NASH through direct triglyceride incorporation. NASH pathogenesis is thought to be related to potentially toxic lipid moieties like saturated fatty acids (SFA), diacylglycerols [3], ceramides and sphingolipids [4-6]. Another lipid particle that might be associated with progression to NASH is cholesterol that can cause precipitation into crystals within the hepatocyte. Its exact role is still under scrutiny [6].

These hepatic fat stores originate either from dietary esterified chylomicrons or, more often, from free fatty acids (FFA). These FFAs can be either the results of a spillover mechanism where they are synthesized from lipolysis of chylomicrons by lipoprotein lipase activation or from a de novo hepatic non-lipid source production mechanisms [7,8]. They can be delivered in excess to the liver from the adipose tissue through a defective inhibition of hormone-sensitive lipase and increased adipose lipolysis promoted by insulin resistance. Within the hepatocyte, insulin resistance is directly induced by some lipid metabolites - mainly SFA and diacylglycerols - and manifests through a
deficit of lipolysis suppression and an excess of de novo lipogenesis through a protein kinase Cε mediated mechanism [9]. An excess of lipids and probably lipotoxic compounds production ensues. Inversely, monosaturated and polyunsaturated FFAs do not seem to induce or aggravate insulin resistance.

At some point, the liver fatty acids excess seems to overcome the protective mitochondrial beta-oxidation and triglyceride production mechanisms from the endoplasmic reticulum and induces a lipotoxic state through metabolic stress with progressive mitochondrial dysfunction [10] and the generation of reactive oxygen species [11].

Other mitochondrial and nonmitochondrial (peroxisome, microsome) enzymatic processes, both toxic and protective, are impaired through long-chain saturated fatty acids like palmitate and stearate excess and seem to participate in this cellular stress state [12-14].

Reactive oxygen species and excess lipotoxins within the hepatocyte lead to the saturation of the endoplasmic reticulum, which responds through a stress state of unfolded protein response (UPR) [15]. This state is characterized by decreased protein and lipid secretion, lipolysis, and initially increased autophagy, which, in the short term, is meant to protect the hepatocyte [16]. In the long term, oxidative stress, NF-kB-mediated inflammatory response [17], insulin resistance [18] and programmed cell death [17,19] are progressively upregulated by the stressed endoplasmic reticulum and perpetual UPR state and become deleterious [20]. This positive loop process leads to hepatic inflammation, autophagy, and repeated programmed cell death and represents the main driver for the passage from simple fatty liver to NASH and, ultimately, cirrhosis.

Autophagy is upregulated in NASH by an excess of unfolded proteins and excess lipid droplets through the mTOR and PI3K pathways, but clearance of the substrate is insufficient. Defective autophagy could explain the persistence of lipotoxic moieties and inflammation within the hepatocyte, which finally leads to programmed cell death and increased hepatic insulin resistance [21]. Insulin resistance independently increases apoptosis through the PI3K, ERK, and MAPK pathways [22] but also through reactive oxygen species generation and mitochondrial permeabilization [23].

Pyroptosis, another type of programmed cell death tied to NF-kB activation, is excessive in individuals with NASH via an excess of gasdermin D signaling that correlates linearly with NAFLD activity and induces a pro-inflammatory environment within the liver [24]. These lipotoxic pathways of inflammation and programmed cell death for the hepatocyte are similar to those that induce activation and fibroblast transformation of hepatic stellate cells [25].

Since lipid storage alone does not always lead to steatohepatitis, factors like genetics [24], microbiota – host interactions, and impaired immune response probably contribute to NASH progression. Specific mitochondrial protein-coding genes [26] but also genes involved in collagen and cholesterol synthesis (Patatin-like phospholipase domain-containing protein 3 - (PNPLA3) [27], insulin signaling (Insulin Receptor Substrate 1 - IRS1), and innate immunity activation (MERTK) inconstantly and heterogeneously associate with NASH. Other external factors like age and nutrient intake (fat or sugars) are important modifiers of the steatosis phenotype; it is accepted that we need to account for extensive interactions with the genotype if we are to model the disease progression [28].

Another target that could explain phenotypic differences in the fatty liver is the host-microbiota interaction. Disturbance of the gut–liver axis by inflammation or bile acid signaling could initiate or aggravate NASH, maybe through excess bacterial lipopolysaccharides within the portal vein. Lipopolysaccharides exposition induces inflammation and autophagy within the healthy liver, but the causal association with NASH is still to be determined.

### Inflammation, hepatocyte death andstellate cell activation and differentiation

Until this point, steatohepatitis is mainly confined to the hepatocyte cytoplasm. Once the endoplasmic reticulum is overwhelmed by lipotoxic by-products and UPR is initiated, and mitochondrial dysfunction with reactive oxygen species formation alongside insulin resistance activates inflammation and programmed death, the process spreads to the nucleus and outside the hepatocyte. Inflammation is initiated by Fas, NF-Kb and TNF receptors, as well as TNF-related apoptosis-inducing ligand receptor [23]. Hepatocyte destruction releases damage-associated molecular patterns (DAMPs) and induces reactive oxygen species that further increase activation of hepatic stellate cells [29] and perpetuates the inflammatory milieu. Through this signaling cascade, macrophages and Kupfer cells residing the liver are recruited and initiate the innate immune response [30,31]. They keep the principal functions, such as phagocytosis, danger signal recognition, cytokine release, antigen processing, and the ability to modulate immune responses [32] but exposure to endotoxins, pathogen-associated molecular pattern (PAMPs), lipopolysaccharides and specific FFAs and cholesterol metabolites limit macrophage differentiation towards fibrinolytic phenotypes [33]. Alongside Kupfer cells and macrophages, activated resident natural killer (NK) cells and natural killer T (NKT) cells produce cytokines that also mediate lymphocyte recruitment [34] and DAMP and PAMP signaling that amplifies apoptosis. In a cascade, pyroptosis continues and cytokines (TNF, IL2, IL6, IL 17) are released, and more effector immune cells are recruited [35]. The profibrogenic effect of innate immunity activation is secondary to TGF-beta secretion by most residing cells (hepatocytes, immune cells, macrophages), which stimulates the transformation of stellate cells into myofibroblasts [36]. Activated endothelial cells mediate this process through the expression of adhesion molecules that facilitate neutrophil and lymphocyte recruitment [37]. Their secretion of IL33 and IL1 superfamily cytokines stimulates the activation of perisinusoidal stellate cells and explains perisinusoidal fibrosis [38]. High titers of antibodies against lipid peroxidation-derived antigens [39] and activated lymphocytes [40] parallel with parenchymal infiltration by lymphocytes suggest the role of adaptive immunity in the perpetuation of NASH and progression to

cirrhosis. Th1/Th2 CD4 + T helper lymphocytes population ratio immunomodulates fibrosis evolution via cytokine synthesis [41]. B and T cell infiltrates correlate with more severe lobular inflammation [42].

This inflammatory process leads to the activation of the main effector of fibrosis progression, the hepatic stellate cells. They represent less than 5% of the healthy liver population that are generally found in the space of Disse and have a role in the storage of lipids and fat-soluble vitamins [43]. Their activation and proliferation modulated by local inflammatory cytokines [31,44, 45,46], lipoprotein lipase pathway [47], circulating noncoding RNA (microRNA and long non-coding RNA) [48,49] and growth factors lead to fibrosis and cirrhosis. When activated, hepatic stellate cells express alpha-smooth muscle actin and transform into myofibroblast. Upon activation, they display a necro-inflammatory activity [50], immunomodulatory, and metalloproteases (TMI1) inhibitory role. They promote fibrosis via extracellular type I and III collagen synthesis [51] and are stimulated by locally produced factors comprising transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF). These myofibroblasts are currently considered to have a central role in liver fibrosis progression [52]. In a minority of patients suffering from NASH, this process will perpetuate, and severe fibrosis and cirrhosis will ensue.

**Conclusion**

Steatohepatitis is a complex and heterogeneous syndrome in which insulin resistance and impaired lipid metabolism seem to induce a constant state of hepatic inflammation that leads to hepatocyte programmed death and activation of tissue repair mechanisms that will eventually conduct to fibrosis and cirrhosis.

**Conflict of Interest**

The authors confirm that there are no conflicts of interest.

**References**

1. Listenberger LL, Han X, Lewis SE, et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proc Natl Acad Sci U S A. 2003;100(6):3077-3082.
2. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology. 2007;45(6):1366-1374.
3. Gorden DL, Ivanova PT, Myers DS, et al. Increased diacylglycerols characterize hepatic lipid changes in progression of human non-alcoholic fatty liver disease; comparison to a murine model. PLoS One. 2011;6(8):e22775.
4. Gorden DL, Myers DS, Ivanova PT, et al. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. J Lipid Res. 2015;56(3):722-736.
5. Garcia-Ruiz C, Mari M, Colell A, Morales A, Fernandez-Checa JC. Metabolic therapy: lessons from liver diseases. Curr Pharm Des. 2011;17(35):3933-3944.
6. Mauer AS, Hirsova P, Maiers JL, Shah VH, Malhi H. Inhibition of sphingosine 1-phosphate signaling ameliorates murine non-alcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol. 2017;312(3):G300-G313.
7. Nelson RH, Basu R, Johnson CM, Rizza RA, Miles JM. Splanchnic spillover of extracellular lipase-generated fatty acids in overweight and obese humans. Diabetes. 2007;56(12):2876-2884.
8. Yamaguchi K, Yang L, McCall S, et al. Diacylglycerol acyltransferase 1 anti-sense oligonucleotides reduce hepatic fibrosis in mice with nonalcoholic steatohepatitis. Hepatology. 2008;47(2):625-635.
9. Chaurasia B, Summers SA. Ceramides - Lipotopic Inducers of Metabolic Disorders. Trends Endocrinol Metab. 2015;26(10):538-550.
10. Pirola CJ, Gianotti TF, Burgueno AL, et al. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut. 2013;62(9):1356-1363.
11. Farrell GC, Haczeyni F, Chitturi S. Pathogenesis of NASH: How Metabolic Complications of Overnutrition Favour Lipotoxicity and Pro-Inflammatory Fatty Liver Disease. Adv Exp Med Biol. 2018;106(1):19-44.
12. Satapati S, Sunny NE, Kucejova B, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. J Lipid Res. 2012;53(6):1080-1092.
13. Rector RS, Thyfault JP, Uptergrove GM, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. J Hepatol. 2010;52(5):727-736.
14. Zhang D, Liu ZX, Choi CS, et al. Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance. Proc Natl Acad Sci U S A. 2007;104(43):17075-17080.
15. Lee S, Kim S, Hwang S, Chernington NJ, Ryu DY. Dysregulated expression of proteins associated with ER stress, autophagy and apoptosis in tissues from nonalcoholic fatty liver disease. Oncotarget. 2017;8(38):63370-63381.
16. Lake AD, Novak P, Hardwick RN, et al. The adaptive endoplasmic reticulum stress response to lipotoxicity in progressive human non-alcoholic fatty liver disease. Toxicol Sci. 2014;137(1):26-35.
17. Luedde T, Schwabe RF. NF-kappaB in the liver--linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2011;8(2):108-118.
18. Lebeaupin C, Vallee D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. J Hepatol. 2018;69(4):927-947.
19. Amir M, Czaja MJ. Autophagy in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 2011;5(2):159-166.
20. Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. Circ Res. 2010;107(5):579-591.
21. Yang L, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 2010;11(6):467-478.
22. Lavallard VJ, Meijer AJ, Codogno P, Gual P. Autophagy, signaling and obesity. Pharmacol Res. 2012;66(6):513-525.
23. Gucciardi ME, Malhi H, Mott JL, Gores GJ. Apoptosis and necrosis in the liver. Compr Physiol. 2013;3(2):977-1010.
24. Xu B, Jiang M, Chu Y, et al. Gasdermin D plays a key role as a pyroptosis executor of non-alcoholic steatohepatitis in humans and mice. J Hepatol. 2018;69(4):773-782.
25. Hernandez-Gea V, Hilscher M, Rozenfeld R, et al. Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy. J Hepatol. 2013;59(1):98-104.
26. Mehta R, Jeiran K, Koenig AB, et al. The role of mitochondrial al genomics in patients with non-alcoholic steatohepatitis (NASH). BMC Med Genet. 2016;17(1):63.
27. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3
rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol. 2014;61(1):75-81.

28. Davis JN, Le KA, Walker RW, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. Am J Clin Nutr. 2010;92(6):1522-1527.

29. Batalle R, Brenner DA. Liver fibrosis. J Clin Invest. 2005;115(2):209-218.

30. Lopez-Navarrete G, Ramos-Martinez E, Suarez-Alvarez K, et al. Th2-associated alternative Kupffer cell activation promotes liver fibrosis without inducing local inflammation. Int J Biol Sci. 2011;7(9):1273-1286.

31. Friedman SL, Arthur MJ. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J Clin Invest. 1989;84(6):1780-1785.

32. Guillot A, Tacke F. Liver Macrophages: Old Dogmas and New Insights. Hepatol Commun. 2019;3(6):730-743.

33. Kazankov K, Jorgensen SMD, Thomsen KL, et al. The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Nat Rev Gastroenterol Hepatol. 2019;16(3):145-159.

34. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43(2 Suppl 1):S54-62.

35. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805-820.

36. Elpek GO. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol. 2014;20(23):7260-7276.

37. Ibrahim SH, Hirsova P, Gores GJ. Non-alcoholic steatohepatitis pathogenesis: sublethal hepatocyte injury as a driver of liver inflammation. Gut. 2018;67(5):963-972.

38. Bhunchet E, Fujieda K. Capillarization and venularization of hepatic sinusoids in porcine serum-induced rat liver fibrosis: a mechanism to maintain liver blood flow. Hepatology. 1993;18(6):1450-1458.

39. Albano E, Mottaran E, Vidali M, et al. Immune response towards lipid peroxidation products as a predictor of progression of non-alcoholic fatty liver disease to advanced fibrosis. Gut. 2005;54(7):987-993.

40. Sutti S, Jindal A, Locatelli I, et al. Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. Hepatology. 2014;59(3):886-897.

41. Shi Z, Waki AE, Rockey DC. Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. Proc Natl Acad Sci U S A. 1997;94(20):10663-10668.

42. Bruzzi S, Sutti S, Giudici G, et al. B2-Lymphocyte responses to oxidative stress-derived antigens contribute to the evolution of nonalcoholic fatty liver disease (NAFLD). Free Radic Biol Med. 2018;124:249-259.

43. Elsharkawy AM, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. Apoptosis. 2005;10(5):927-939.

44. Hellerbrand C, Stefanovic B, Giordano F, Burchardt ER, Brenner DA. The role of TGFbeta1 in initiating hepatic stellate cell activation in vivo. J Hepatol. 1999;30(1):77-87.

45. Kawada N. Cytoglobin as a Marker of Hepatic Stellate Cell-derived Myofibroblasts. Front Physiol. 2015;6:329.

46. Elpek GO. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol. 2014;20(23):7260-7276.

47. Teng KY, Ghoshal K. Role of Noncoding RNAs as Biomarker and Therapeutic Targets for Liver Fibrosis. Gene Expr. 2015;16(4):155-162.

48. Yang JJ, Tao H, Deng ZY, Lu C, Li J. Non-coding RNA-mediated epigenetic regulation of liver fibrosis. Metabolism. 2015;64(11):1386-1394.

49. Cortez-Pinto H, Baptista A, Camilo ME, de Moura MC. Hepatic stellate cell activation occurs in nonalcoholic steatohepatitis. Hepatogastroenterology. 2001;48(37):87-90.

50. Fallowfield JA. Future mechanistic strategies for tackling fibrosis—an unmet need in liver disease. Clin Med (Lond). 2015;15 Suppl 6:s83-87.

51. Mederacke I, Hsu CC, Troeger JS, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun. 2013;4:2823.