

# Immunological and molecular mechanisms in NAFLD

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## ABSTRACT

Chronic metabolic inflammation, maintained by numerous proinflammatory mediators, links the metabolic syndrome to NASH. Epigeal changes that accelerate the synthesis of proinflammatory mediators in response to nutritional factors and life style are not fully known. Numerous proinflammatory transcription pathways have been described where nuclear factor kappa B plays a critical role in stimulating proinflammatory gene promoter region interdependent with epigenetic machinery. Of the many cytokines, adipokines, myokines involved in the evolution of NAFLD from simple steatosis to NASH and cirrhosis, the most important is the balance between proinflammatory cytokines  $TNF\alpha$  and IL 6 on one hand and adiponectin on the other hand, these factors being produced by Kupffer cells, hepatic stellate cells and inflammatory hepatocytes. Starting from the classical "two hit" theory of the pathogenesis of NAFLD, recent studies have revealed the important role of free cholesterol, diacylglycerols and ceramides in the induction of insulin resistance by stimulating sterol-element-binding protein 1c and thus stimulating de novo lipogenesis. The important roles of mitochondrial dysfunction, oxidative stress endoplasmic reticulum, inflammasomes, hepatocyte apoptosis and necrosis have also been highlighted.

**Key words:** non-alcoholic fatty liver disease (NAFLD), pathogenic pathways, molecular disorders

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents the most frequent cause of liver disease in developed countries and has the potential to evolve towards cirrhosis and hepatocellular carcinoma (1,2). Nonalcoholic steatohepatitis (NASH) is considered to be the progressive subtype of NAFLD. NAFLD/NASH is considered to be the liver expression of the metabolic syndrome, which is characterized by obesity, altered glucose tolerance/diabetes mellitus, dyslipidemia and hypertension. The clinical and physiopathological characteristics have been for the most part clarified, but the mechanisms of progression are incompletely described.

The important pathological features of NASH are: hepatocytic necrosis, inflammation, liver fibrosis, hepatocyte ballooning and development of Mallory-Denk bodies.

Pathogenesis of NAFLD/NASH is influenced by lifestyle, nutritional factors, genetic influences and immunological mechanisms.

The classical pathogenic theory implies a first hit, in which accumulation of fat occurs in the liver, while during the second hit the main role is played by oxidative stress and cytokines. Regarding the first hit, lipidomic studies have shown that free cholesterol, free fatty acids, diacylglycerides or ceramides are lipotoxic molecules that accumulate selectively in NASH. Besides these, an important role is played by the depletion of polyunsaturated fatty acids and a decrease of hepato-protective eicosanoids (3,4).

### *Initiation of inflammation in nonalcoholic fatty liver*

Inflammation that affects or infiltrates the liver in NASH may initiate outside the liver, especially in the visceral fatty tissue. The important consequence is the release of chemokines and cytokines, especially macrophage chemotactic protein 1 (MCP-1) and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ). Recent studies have shown that mice fed with a cholesterol-enriched diet present an increased transcription of cytokines and an increased macrophage level in fatty tissue after 6-16 weeks, as well as in the liver after 16-26 weeks (5).

A significant increase of serum MCP-1 levels is an expression of the systemic and fatty inflammatory status in the metabolic syndrome (6). MCP-1 and its receptor CCR2 are important molecules because, like in the case of NF-KB and C-Jun N-terminal Kinase (JNK), they unite the inflammatory response with insulin resistance (7).

### *TNF $\alpha$*

The increased expression of TNF $\alpha$  and of its type 1 receptor (TNFR1) has been proven in patients with NAFLD/NASH, being much more evident in subjects with NASH than in those with simple steatosis (8). It is interesting to note that increased TNF $\alpha$  expression correlated with fibrosis. Patients with NASH present an augmented apoptosis phenomenon compared with simple steatosis, which correlates with nuclear factor KB, with TNFR1, inflammatory activity and grade of fibrosis (9).

It has also been established that TNF $\alpha$  modulates systemic and hepatic sensitivity to insulin. Hotamisligil and collab (10) were the first to remark the relationship between obesity, increased expression of TNF $\alpha$  and insulin activity, establishing the concept of inflammation in obesity and expression of TNF $\alpha$  in adipocytes.

In human pathology, an increased adipocyte

expression of TNF $\alpha$  has been shown in obese subjects, as well as the correction of this expression after weight loss (11). Both TNF $\alpha$  and its receptor correlate with body mass index (12). TNF $\alpha$  stimulates phosphorylation of insulin receptor substrate 1 (IRS-1), thus decreasing its affinity for insulin. It also stimulates the I $\kappa$ B Kinase  $\beta$  (IKK $\beta$ ) pathway, which activates inflammatory pathways and blocks insulin receptor signalling.

Mitochondrial dysfunction represents a central mechanism that links obesity to metabolic complications (13). Decrease in the activity of the respiratory chain leads to accumulation of reactive oxygen species (ROS), which oxidate fatty deposits forming lipid peroxidase products, which in turn produce necrosis, inflammation and fibrosis. Visfatin (NAMPT-nicotinamide phosphoribosyl transferase) is a molecule with an extracellular cytokine-like activity and an intracellular enzymatic-type activity. It has pro-inflammatory activity, inducing TNF $\alpha$  and IL6. Patients with NAFLD present increased visfatin levels, while weight loss is associated with the decrease of plasma and hepatic levels (14). Formation of mitochondrial ROS determines lysis of mitochondrial DNA and respiratory chain polypeptides, induces NF-KB activation and hepatic synthesis of TNF $\alpha$ .

### *Adiponectin (AD)*

Adiponectin is a protein produced exclusively by adipocytes.

Unlike other hormones derived from fatty tissue, production and concentrations of AD are decreased in obese subjects. The AD gene is located on chromosome 3 and encodes a 244-aminoacid polypeptide. The C-terminal half has a globular domain with sequences homologous to protein C1q.

The expression of the AD gene in white fatty tissue is decreased by obesity, glucocorticoids,  $\beta$ -adrenergic agonists and TNF $\alpha$  and is increased by adrenalectomy, insulin-like growth factor-1 (IGF1) and leanness.

In *ob/ob* leptin deficient obese mice, a 2-fold decrease of AD levels and a 10-fold decrease of gene expression have been observed compared to strain-matched controls (15).

Experiments have also shown that adiponectin-knockout mice (KO) have a decreased plasma clearance of fatty acids, a decreased level of fatty-acid transport protein 1 mRNA in muscles, increased levels of TNF $\alpha$  mRNA in fatty tissue and increased plasma TNF $\alpha$  levels (16).

Moreover, although these KO mice had normal glucidic tolerance and received a normal diet, once they moved to a diet rich in fats they developed insulin resistance and decreased expression in the muscles of

phosphatidyl inositol 3 kinase, which is the substrate of the insulinic receptor (IRS1).

In humans, normal plasma levels for AD are 0.5-30 microgr/ml, approximately 1000 times higher than leptin and insulin levels. Many clinical studies have shown a decrease of AD plasma levels in obese subjects compared to normal-weight individuals (17).

Adiponectin is decreased in diabetics compared to non-diabetics, is negatively correlated with serum insulin levels, and inversely correlates with triglyceride levels and with plasma concentrations of glucose before and after meals (18,19).

Stimulation of PPAR $\gamma$  with agonists such as thiazolidine-diones increases adiponectin plasma levels, while PPAR $\gamma$  agonists such as metformin had no effect (20).

The anti-inflammatory effect of adiponectin has been described in endothelial cells (21). In this experiment, adiponectin inhibits endothelial adhesion molecules induced by TNF $\alpha$ . Adiponectin produces anti-inflammatory effects by inducing IL-10 and IL-1-receptor antagonist in different cell types and inhibits IL6 and interferon  $\gamma$  (22).

There are two adiponectin receptors: adipoR1 present especially in skeletal muscles and adipoR2 present predominantly in the liver.

Administration of recombinant adiponectin determines an activation of AMP-activated kinase (AMPK) with remission of hyperglycemia and of insulin resistance in diabetic ob/ob mice (23).

Hepatic stellate cells (HSC) express type 1 and 2 receptors, through which adiponectin inhibits proliferation, migration and expression of TGF  $\beta$ 1, the main activator of extracellular matrix synthesis.

Adiponectin improves NASH and liver fibrosis by suppressing activation of Kupffer and hepatic stellate cells. In serum macrophages, adiponectin suppresses synthesis of TNF $\alpha$  and IL-6 stimulated by LPS and generates IL10 expression. Attenuation of pro-inflammatory cytokine production is due to an attenuation of NF-KB translocation to the nucleus (24). The anti-inflammatory effect of adiponectin in macrophages involves the toll-like receptor 4 (TLR-4) signalling pathway.

Transformation of HSC into myofibroblasts represents the main factor in initiating the process of fibrosis in liver injury. Through the two receptors present in HSC, adiponectin inhibits proliferation and migration of HSC induced by platelet-derived growth factor and inhibits MCP-1 production through AMPK-dependent mechanisms (25).

In the study of Targher et al (26), 60 NAFLD patients diagnosed by liver biopsy presented decreased plasma

adiponectin levels compared to the witness group. Adiponectin levels correlated with the degree of liver steatosis, inflammation and fibrosis.

By activating AMPK, adiponectin inhibits the expression of sterol regulatory element-binding protein 1c (SREB-1c), a transcriptional factor that regulates synthesis of cholesterol and lipids. The decrease in SREB-1c leads to a sub-expression of genes involved in lipogenesis (27). On the other hand, adiponectin stimulates PPAR $\gamma$ , thus amplifying the genes that encode enzymes involved in oxidation of fat, in reducing lipid synthesis and in preventing hepatic steatosis (28).

### *Leptin*

Leptin is expressed mainly in the fatty tissue, decreases appetite and increases energy expenditure, so that it limits fatty accumulation in the fatty tissue and reduces lipotoxicity. By increasing free fatty acids (FFA) offered to the liver, leptin induces dephosphorylation of the insulin receptor substrate 1 (IRS1) in order to prevent fatty accumulation at this level (29). Hepatic stellate cells express leptin, which shows that leptin is involved in fibrogenesis and progression of liver disease (30).

Studies performed in NAFLD patients have provided different results than those in laboratory animals. In such a study (31), patients with biopsy-proven NAFLD presented leptin levels that did not correlate with the grade of steatosis, so that leptin levels could not differentiate between patients with simple steatosis and those with NASH. During follow-up, patients with NASH and increased leptin levels presented a significant decrease of aminotransferases.

In the study of Huang XD et al (32), patients with biopsy-proven NAFLD presented higher serum leptin levels than those in the control group, and this negatively correlated with serum levels of the soluble leptin receptor (OB-Rs). Leptin levels did not correlate with BMI or HOMA. Decrease of OB-Rs suggests an increase in the resistance of peripheral tissues to leptin activity.

Recent data indicate that leptin infusion improves liver steatosis through reduction of hepatic triglyceride synthesis and improvement of IR in lipodystrophy model mice (33).

The hormone exerts pro-inflammatory and pro-fibrogenic effects in murine liver exposed to hepatotoxic substances and up-regulates production of pro-inflammatory and pro-angiogenic cytokines in HSC (34).

In children with NASH (35), increased leptin levels correlated with the degree of liver injury.

### ***Interleukin 6***

Interleukin 6 (IL6) is a cytokine that plays an active role in the humoral and cellular immune response. It is produced by various cells, among which adipocytes and macrophages of the fatty tissue. IL6 displays its activity after binding to its receptor IL-6RB, forming glycoprotein 130 (GL 130). It increases lipolysis and serum FFA levels and inhibits the lipoprotein lipase (36).

In obese individuals, 15-30% of the total IL-6 is produced in the fatty tissue. Increase of IL-6 levels positively correlates with IR and with the cardiovascular risk (37).

The mechanism of IR is promoted by induction of the JAK/STATs activating pathway, which leads to transcription of several genes, such as the suppressor of cytokine signalling (SOCS) which inhibits IRS phosphorylation (38).

In a recent study (39), interleukin 6 was found to be increased in obese subjects compared to the control group and also in individuals with the metabolic syndrome.

Due to its effect on IR and on regulation of inflammation, which represent factors involved in NAFLD, IL-6 has been proposed as a factor that generates NAFLD (40).

### ***Interleukin 10***

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that improves hepatocellular injury (41). It has been reported that endogenous IL-10 does not improve IR, but it prevents hepatic steatosis in laboratory animals (42).

In a different NAFLD laboratory model, IL-10 inhibition with neutralizing antibodies worsened IR by increasing TNF $\alpha$  and IL-6 expression in the liver (43).

### ***Resistin***

Resistin is a cytokine also secreted by adipocytes, with a role in development of insulin resistance.

In the study of Pagano C et al (44), the levels of resistin, adiponectin and leptin were determined in patients with NAFLD, in obese subjects without liver injury and in a witness group. Those with NAFLD and obese subjects had decreased adiponectin levels, while leptin was increased only in the obese. On the other hand, resistin did not correlate with C reactive protein, BMI, HOMA, glycemia or aminotransferases; it positively correlated with the histological activity score.

In another study (45), the resistin polymorphism +299 G/A was determined in patients with type 2

diabetes mellitus. Plasma resistin level was increased in patients with type 2 diabetes mellitus and NAFLD. The frequency of the AA genotype in site +299 of resistin conferred a 1.8 risk for type 2 diabetes mellitus and NAFLD, and a 2.05 risk for obesity compared to genotype GG.

Resistin is a potential link between obesity and insulin resistance or type 2 diabetes mellitus. Exposure of rodents to resistin results in a decrease of response to insulin. This is partly due to up-regulation of suppression of cytokine signaling (SOCS)-3, which interferes with activation of the insulin receptor substrate (IRS1) (46).

In the study of Boyraz M et al (47) on patients with NAFLD, it was noticed that leptin and RBP4 were higher in obese with NASH than in obese without NASH, while resistin negatively correlated with adiponectin.

Fatty tissue is no longer seen as a triglyceride-storing depot, but as an endocrine organ that produces and secretes bioactive factors known as adipokines. These consist in cytokines and chemokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukins, monocyte chemoattractant protein 1 (MCP1), leptin, adiponectin, visfatin, apelin, dipeptidyl peptidase-4 and others.

### ***ADIPONUTRIN (Patatin-like-phospholipase domain-containing protein 3) (ADPN)***

ADPN/PNPLA3 belongs to the patatin-like phospholipase family. It is expressed in the liver and adipose tissue and possesses acyl hydrolase activity. ADPN expression is increased by carbohydrate overload and Western type diet (48) and it has a lipase activity against triglyceride and acylglycerol transacylase activity. It is involved in energetic mobilization and storage of fat. The adiponectin genotype was identified by genome wide association as a determinant of hepatic triglyceride accumulation. Identification of a SNP in the adiponectin gene (vs 738409 C $\rightarrow$ G coding the I148M) that generates a protein variant is the strongest genetic determinant of the fatty liver and AST levels (49). I148M affects hepatic steatosis independent of weight, dyslipidemia and insulin resistance. The risk of NAFLD is 3.3 times higher in subjects with GG vs738409 genotype compared to CC genotype. In a recent study (50) based on a group of NAFLD patients and a control group, vs738409 GG genotype was 14% in patients versus 3% in the control group. The study showed the association of this polymorphism with the degree of liver steatosis and fibrosis and also Fas ligand proapoptotic molecule. All these studies reveal the role of adiponutrin in hepatic triglyceride accumulation.

### ***Kruppel-like factor 6 (KLF 6)***

KLF 6 is a multifunctional transcriptional factor with a role in glucose and lipid homeostasis. KLF 6 induces adipogenesis in preadipocytes and fibroblasts and interferes with PPAR $\alpha$ . Functional polymorphism of the KLF 6 gene that increases the generation of alternative KLF 6 antagonist protects patients with advanced NAFLD (51). A recent study (52) investigated on laboratory animals and a control group of 28 patients with NAFLD the mechanism of KLF 6 and the correlation with carbohydrate and lipid metabolism. Insulin resistance is mediated partly by PPAR $\alpha$ , a xenobiotic sensor that regulates lipid and hepatic steatosis, lipoprotein synthesis and hepatic gluconeogenesis. PPAR $\alpha$  is activated by excess fatty acids (FA). Micro RNA 10b (miRNA10b) inhibits PPAR $\alpha$  protein expression. Laboratory animals (mice) with partial or complete depletion of KLF 6 have reduced hepatic steatosis and improved carbohydrate and insulin tolerance. In hepatocytes, KLF 6 deficiency reduces the expression of genes regulated by PPAR via miRNA10b, repressed by KLF 6. In patients with NAFLD and advanced stage of inflammation, expression of KLF 6 is increased and the miRNA is significantly decreased. The study supports the idea that KLF 6 is a new regulator of glucose and lipid metabolism in fatty liver.

### ***Hepassocin (HPS)***

HPS is a hepatokine whose expression is increased in liver regeneration, playing an important role in hepatocyte proliferation through an autocrine mechanism (53). Blocking HPS causes inhibition of cell growth, this hepatokine being lower in patients with hepatocellular carcinoma (HCC) (54). A recent clinical and experimental study (55) evaluated the role of HPS in NAFLD. Subjects with NAFLD had higher serum concentrations of HPS vs those without NAFLD. HPS overexpression increases lipid accumulation and liver NAFLD activity score, while deletion of HPS improves hepatic steatosis induced by a rich fat diet and NAFLD activity score in laboratory mice. In addition, the use of oleic acid, a steatogenic reagent, in laboratory animals increases HPS expression in hepatocytes. The authors also used HepG2 cell line to demonstrate that overexpression of HPS induces lipid accumulation by extracellular signal-regulated kinase pathway 1/2 (ERK1/2), while deletion of HPS decreases lipid accumulation induced by oleic acid. The insulin activates ERK1/2 pathway to increase the transcriptional activity of sterol regulatory element binding protein 1 (SREBP1), so HPS could play a role in regulating insulin sensitivity and metabolic homeostasis.

### ***Fetuin A***

Fetuin A ( $\alpha_2$  Heremans-Schmid glycoprotein) is an endogenous inhibitor of insulin receptor tyrosine kinase (56). Administration of fetuin A in rodents inhibits phosphorylation of insulin receptor and its substrate 1 thereof. A number of studies support that fetuin A is involved in the pathophysiology of insulin resistance and type 2 diabetes (57,58). The experimental data were also correlated with epidemiological evidence. The study of Stefan N et al (59) analyzed a sample of 2164 individuals, diabetes free at baseline, followed for 7 years and for which anthropometric and metabolic data were recorded. Of these, 849 developed diabetes. Plasma levels of fetuin A were positively associated with the risk of diabetes (RR for 10  $\mu$ g/ml 1.04 [1.03-1.06]). Fetuin A expression and its plasma levels were found elevated when there was accumulation of fat in the liver (60) and in conditions of metabolic syndrome (61). Based on these data and on the observation that fatty liver predisposes to diabetes type II (62), it may suggest that fetuin A is a mediator of type II diabetes induced by fatty liver.

### ***Fibroblast growth factor 21***

Human fibroblast growth factor (FGF21) belongs to a family of growth factors together with FGF 19, FGF-23, with metabolic-like properties, interacting with their own receptors. FGF21 expression is increased in the liver and is an important regulator of glucose and lipid metabolism. Therapeutic intervention with recombinant FGF21 causes a decrease in serum glucose and triglycerides (TG) in ob/ob and ad/db diabetic mice (63). Systemic administration of FGF21 results in significant reduction in hepatic steatosis in mice with diet-induced obesity (64). Several studies have shown that PPAR $\alpha$  and its agonists mediate therapeutic benefits by stimulating liver production of FGF21. Some epidemiological studies showed elevated serum FGF21 levels in obese subjects with hypertriglyceridemia and in patients with type II diabetes (65). A recent study (66) examined FGF21 levels in 224 patients with NAFLD compared with a control group. They determined the hepatic expression of FGF21 mRNA, besides lipid profile and markers of liver injury. FGF21 serum levels were significantly increased in patients with NAFLD vs control group ( $p < 0.01$ ). In the liver tissue, FGF21 mRNA was raised in the first group and correlated with the degree of steatosis and intrahepatic triglyceride. This study supports the role of serum FGF21 as a potential biomarker for NAFLD.

### ***Retinol binding protein 4 (RBP4)***

RBP4 is a new adipokine. The principal source is the liver and adipocytes. It has a role in insulin resistance and metabolic syndrome. RBP4 levels are elevated in plasma of patients with IR and correlate with disease severity (67). In recent studies, elevated plasma RBP4 was correlated with hepatic steatosis (68).

Xia et al (69) demonstrated on Hep G2 cells that RBP4 stimulates lipogenesis in hepatocytes by up-regulation of PPAR-coactivator 1- $\beta$ .

### ***Visfatin (NAMPT)***

Visfatin (VF) is produced and released not only by adipocytes, but also by inflammatory cells such as macrophages. VF also has a nicotinamide phosphoribosyl transferase-like enzymatic activity.

Visfatin has been proposed in recent years as a marker of endothelial dysfunction with a role in progression of atherosclerotic processes.

Serum VF levels correlate with inflammatory markers such as IL-6, CRF and MCP-1.

VF has been reported to be associated with obesity (70).

### ***Irisin***

Irisin is a recently discovered myokine involved in regulation of the energetic homeostasis and of the metabolism, released during and after physical effort.

Decreased irisin levels have been shown to be associated with increased intrahepatic triglycerides determined by H1-magnetic resonance spectroscopy (71).

In another study (72), serum irisin levels were determined in patients with biopsy-proven NAFLD compared to a control group with normal-weight and obese individuals. Although serum irisin level was similar in subjects with steatosis and with NASH, it presented a statistically significant correlation with portal inflammation.

### ***Immunological mechanisms of inflammation in NASH***

Inflammation is a risk factor seen in obesity and is due to dysfunction of the fatty tissue which has been shown to be an active endocrine organ capable of secreting hormones, cytokines, chemokines. Anytime the mass of fatty tissue is in expansion, the balance between pro-inflammatory (TNF $\alpha$ , IL-6) and anti-inflammatory cytokines (adiponectin, IL-10) is disturbed in favor of the former (73).

Besides the fatty tissue, hepatic cells play an impor-

tant role. Hepatocytes present lesions of ballooning, Mallory bodies, apoptosis. The latter activates caspases, which cleave a series of substrates among which CK-18. Fragments of CK-18 are increased in patients with NAFLD compared to witnesses (74).

Other hepatic cells that can play a pro-inflammatory role are Kupffer cells (KC), macrophage cells, NK cells, T cells and hepatic stellate cells. Cytokines and chemokines are responsible for secreting macrophage cells, neutrophils, lymphocytes in the inflammatory infiltrate in NASH.

Free cholesterol, FFA, diacylglycerides produce hepatocyte injury by directly or indirectly activating JNK-NF-K $\beta$  and determine mitochondrial dysfunction with stimulation of ROS production. It has been demonstrated that cholesterol determines Fas-mediated apoptosis by depletion of mitochondrial GSH. TNF $\alpha$  is more a consequence than a cause of liver inflammation in NASH. The increased level of TNF $\alpha$  in obese subjects originates in the macrophages of the inflamed fatty tissue (75). TNF $\alpha$  levels do not differentiate NASH from simple steatosis.

Oxidative stress is another pro-inflammatory pathway in NAFLD. There are multiple sources of pro-oxidants in NASH such as mitochondria (uncoupling of oxidative phosphorylation with nitophagia as a consequence), ER dysfunction (over-expression of the P450 2E1 and 4A cytochrome), inflammasomes (NLRP3 or cryopyrin can recruit pro-caspase 1) (76,77).

Cell death triggers inflammation and immune response by pro-inflammatory DAMPS (damage-associated molecular patterns) such as HMGB1, heat shock proteins, mitochondrial products and mitochondrial DNA. DAMPS are recognized and bound by TLR (1, 2, 4, 9 on KCGs, hepatocytes and HSCs), and this activates transcription factors such as NF-K $\beta$ , AP-1 (via JNK) and interferon-responsive factors (*figure 1*).

### ***The role of immune cells in NASH***

Kupffer cells found in the sinusoid contribute to hepatocyte necrosis through Toll-like receptor 9 and IL-1 $\beta$  (78). TNF $\alpha$  produced by KCS is essential for progression of fibrosis in NASH. Neutrophils are activated by hepatocyte necrosis and perpetuate steatohepatitis by releasing proinflammatory cytokines and myeloperoxidase which is a source of free radicals which in turn increase oxidative hepatocellular damage (79). The increase of neutrophil / lymphocyte ratio increases the probability of progression of steatosis to NASH and fibrosis emphasis (80). Dendritic cells (DCs) are antigen presenting cells that support the adaptive immune response. They induce a pro-

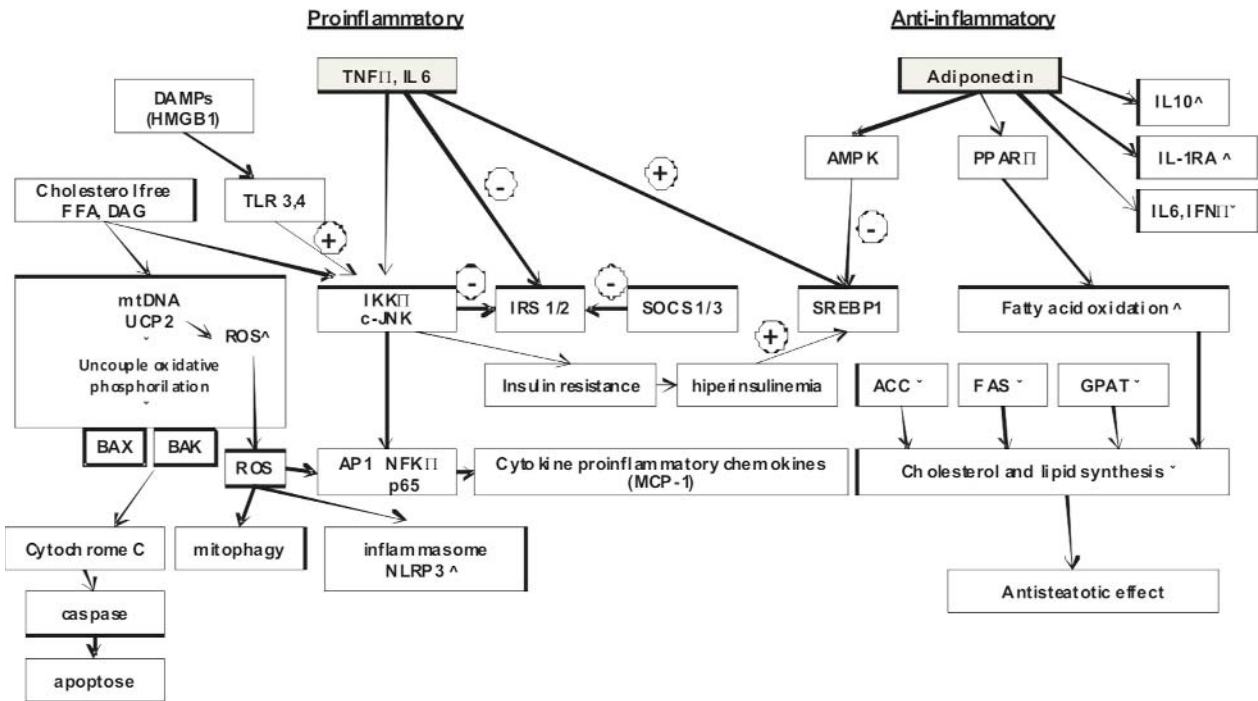


Figure 1 - Immunological mechanisms of inflammation in NASH

SOCS – suppressors of cytokine signaling; AR1 – activator protein 1; DAMPs – danger associated molecule patterns; TLR – Toll-like receptor; MHGM1 – high-mobility group gel box 1; NLRP3 inflammasome – nucleotide-binding domain, leucine-rich repeat containing; AMPK-AMP – activated kinase; ACC – Acetyl Coenzyme A carboxylase; FAS – Fatty acid synthase; GPAT – Glycerol- 3-phosphate acyltransferase; SREBP-1c – sterol regulatory element binding protein 1c; BAX, BAK – proapoptotic proteins

inflammatory, as well as anti-inflammatory immune response. DCs, according to subtype and activation status, produce IL6, IL-12, TNF $\alpha$  and IL-10. Henning et al (81), using mice model of NASH by methionine-choline deficient diet, noted a 3-4 fold increase in CD11c + MHC + DCs times compared to the control group, in early stages of NASH. At the same time, Kupffer cells, monocytes, neutrophils and CD8T increased while NK, NKT, CD4T and B cells decreased. Isolation and in vitro cultivation of DCs 6 weeks from the onset of NASH showed inflammatory features with increased production of IL-6, TNF, and MCP1. Henning et al (81) following histological lesions, demonstrated that DC increase IL-10 production, limiting inflammation and fibrogenesis.

### CONCLUSIONS

Despite the many published studies, it is not known exactly who will develop NAFLD lesions, or out of these NAFLD subjects who will evolve toward NASH and cryptogenic cirrhosis. Nutrition rich in calories and lipids, obesity, insulin resistance, type 2 diabetes mellitus and hypoadiponectinemia are metabolic

determinants of NASH.

The pathogenic pathways which lead to these injuries involve pro-inflammatory pathways that are initiated in hepatocytes subjected to mitochondrial dysfunction, inflammasomes, ER stress, oxidative stress, as well as in the fatty tissue which becomes a pro-inflammatory organ, both locations producing increased quantities of adipocytokines, cytokines and chemokines.

Recent studies have revealed the important role of apoptosis at the hepatic level, but also of T cell changes (Tregs, CD4T, CD8T) with role in hepatic inflammation and the anti-inflammatory and anti-apoptotic role of dendritic cells.

The understanding of these pathogenic pathways will lead to new therapies for this liver disease.

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