Non-alcoholic fatty liver disease (NAFLD): An Immunologist's Perspective

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Abstract*:* The present work was designed to review the literature about immunopathogenesis of Nonalcoholic Fatty Liver Disease. We found that, NAFLD constitutes one of the three major causes of cirrhosis and liver transplantation, given the possible evolutive course of this disease, and can also be associated with the occurrence of a hepatocellular carcinoma. Immune and inflammatory pathways have a central role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Both the innate and adaptive immune systems contribute to the development of NAFLD. Pathogen- associated molecular patterns and danger-associated molecular patterns are known to activate a variety of pattern- recognition receptors that result in inflammation. **Conclusion:** The pathogenesis of NAFLD is complex and implicates cross-talk between different metabolically active sites, such as liver and adipose tissue. Obesity is considered a chronic low-grade inflammatory state and the liver has been recognized as being an "immunological organ". The complex role of the immune system in the pathogenesis of NAFLD is currently raising great interest, also in view of the possible therapeutic potential of immunotherapy in NAFLD.
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1. Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) is a condition defined by excessive fat accumulation in the form of triglycerides (steatosis) in the liver (> 5% of hepatocytes histologically). A Subgroup of NAFLD patients have liver cell injury and inflammation in addition to excessive fat (steatohepatitis) ***(Chalasani et al., 2012).***

NAFLD is characterized by a typical sequence of disease stages. Early stages involve lipid accumulation (steatosis) and inflammation, which may proceed to chronic inflammation and compensatory tissue repair, leading to accumulation of collagen and scarring (fibrosis or cirrhosis) ***(Tilg et al., 2010).*** Liver cirrhosis is associated with progressive loss of organ function and forms the basis for hepatocellular carcinoma (HCC) development. At a certain stage, liver cirrhosis and HCC are non-reversible often leaving organ transplantation as the only therapeutic option ***(Wree et al., 2013***.

Pathogenesis of NASH is complex and implicates cross-talk between different metabolically active sites. The initial "two hits" hypothesis described insulin resistance as "first hit" that leads to hepatic steatosis and is followed by a "second hit" driven by oxidative stress, which in turn leads to the development of steatohepatitis and fibrosis ***(Day and James 1998).*** This model has been expanded in a "multiple parallel hits" hypothesis in which a number of different processes may contribute to liver inflammation.

Currently there is no approved pharmacological treatment available for NASH. The emerging role of disturbances of the immune system in the pathogenetic mechanisms of NASH opens perspectives for new potential therapeutic options through immuno-regulation Preliminary trials have used the anti-CD3 moAb, which is able to prevent induction and progression of inflammatory and autoimmune diseases ***(Von Boehmer and Daniel 2013).***

Aim of the Work

The aim of this essay is to declare the role of the immune system in the pathogenesis, progression and management of NAFLD.

Nonalcoholic Fatty Liver Disease

NAFL:

Steatosis can be diagnosed either using a liver biopsy (≥5–10% of hepatocytes exhibit macroscopic steatosis) or by imaging techniques such as ultrasound (US) (degree of brightness of liver parenchyma, semi-quantitative) or proton magnetic resonance spectroscopy (1H-MRS). Steatosis on US and 1H-MRS does not, however, exclude NASH, as they merely quantify steatosis. Histologically, NAFL encompasses any degree of steatosis alone or steatosis with lobular inflammation but without ballooning **(Kleiner and Brunt, 2012).**

NASH:

NASH can only be diagnosed by liver biopsy. The presence of ballooning injury is the key to the diagnosis. Ballooning degeneration is a form of hepatocyte cell death where the cells increase in cell

size (balloon). It colocalizes initially amidst steatosis in zone 3 near the centrilobular veins. Steatosis and inflammation can be observed to any degree. Fibrosis is not required to make the diagnosis of NASH but is often present. It also begins in zone 3 as delicate strands of collagen **(Kleiner and Brunt, 2012).**

Incidence

There is no precise data on the incidence rates for NAFLD. This is partly due to the fact that NAFLD is usually a silent disease which is discovered incidentally. Nevertheless, given that the prevalence of obesity in adults has almost doubled since the early 1960s (1962–48% vs 2010–75%), the incidence of obesity-related NAFLD has almost certainly increased. In Asia, a few studies have reported an annual incidence of 3–5%; however, the exact incidence rates for NAFLD from different regions of the world are not available **(Paredes *et al*., 2014).**

Prevalence

The prevalence of NAFLD is estimated to range from 27–34% within the general population **(Lazo *et al*., 2013).** However, in certain subpopulations the estimated prevalence of NAFLD is significantly higher. In fact, the prevalence of NAFLD in the morbidly obese ranges from75% to 92% while the prevalence in patients with type 2 diabetes is estimated to be between 60% and 70% **(Younossi *et al*., 2012).** Furthermore, ethnic differences in the prevalence of NAFLD have been reported. In this context, Hispanic Americans have the highest prevalence of 45%, while African Americans have the lowest prevalence of 24% and European American has a prevalence of 33% **(Pan and Fallon, 2014).**

In addition, approximately 3–5% of the US population is estimated to have NASH which is the progressive form of NAFLD. Given the requirement for histologic confirmation of NASH, the true prevalence of NASH in the general population is not available **(Chalasani *et al*., 2012).**

Pathogenesis:

There are several pathways that may lead to the accumulation of hepatic fat. These include increased hepatic lipogenesis, decreased expulsion of hepatic lipid stores, and/or diminished oxidation of free fatty acids in the liver **(Yilmaz, 2012).** These processes promote lipid deposition within the hepatocyte leading to the development of macrovesicular steatosis. Nevertheless, this process of hepatic steatosis alone is not enough to cause progressive liver injury. The damage is then brought about by a number of additional hits such as oxidative stress, which is responsible for lipid peroxidation in the cell membrane **(Paredes *et al*., 2012).**

Adipose Tissue Dysfunction

Adipose tissue is not inert as traditionally thought but has an endocrine function and secretes hormones (adipokines) such as leptin and adiponectin. Obesity-related adipocyte hypertrophy and/or insulin resistance result in an imbalance of adipokines that may profoundly affect not only the adipose tissue itself but also the liver. Adipose tissue contributes to the maintenance of low grade inflammatory states by producing pro-inflammatory cytokines: serum levels of IL-6 and adipocytes expression of TNF-α are increased in obese patients and subsequently decline following weight loss. Furthermore, increased expression of inflammatory genes and macrophages activation in the visceral and subcutaneous adipose tissue of patients with NAFLD correlates with progression from simple steatosis to NASH and fibrosis **(du Plessis *et al*., 2015).** Leptin is a 16-kDa an-orexigenic hormone with proinflammatory actions that prevents lipid accumulation in non-adipose sites; in the liver, this is achieved by lowering the expression of SREBP-1. However, leptin increases in obese subjects as a consequence of leptin resistance and its profibrogenic role has been demonstrated in various in vitro and animal models **(Tsochatzis *et al*., 2008).** Leptin activates hepatic stellate cells through the hedgehog and mTOR pathways. It was suggested that Kupffer cells are targeted by leptin and are stimulated to produce TGF-β1 and subsequently activate hepatic stellate cells **(Aleffi *et al*., 2011).**

Genetic Determinants

Genetic variants, especially in the form of single nucleotide polymorphisms, influence hepatic FFAs flux, oxidative stress, response to endotoxins and cytokine production and activity, and are determinants of NAFLD development and progression. Genome-wide association studies have established the role of patatin-like phospholipase 3 (PNPLA3) single nucleotide poly-morphisms in the development and progression of NAFLD, in particular the I148 M (rs738409 C/G) variant. PNPLA3 gene encodes for a protein called adiponutrin which has significant homologies to enzymes implicated in lipid metabolism processes and could exert a lipolytic activity on triglycerides **(Anstee and Day, 2013).**

Epigenetic Factors

Clinical studies investigating epigenetic reprogramming in NASH are just beginning to emerge. Epigenetic modifications are stable changes at transcriptional level, such as DNA methylation, histones modifications and activity of microRNAs (miRNAs), which do not alter the basic DNA sequences and contribute to the cell homeostasis exhibiting a high degree of developmental and environmentally driven plasticity **(Zeybel *et al*., 2013).**

It has been hypothesized that the disruption of this balance may determine an increased susceptibility for NAFLD **(Podrini *et al*., 2013).**

Diagnosis:

1. **Liver Enzyme Tests**

Aminotransferases (ALT, AST) are commonly used to screen for liver disease. Reference values for ALT and AST are dependent on gender and age. Since these enzymes tend to fluctuate, significant liver disease may be present in patients with "normal" liver enzymes. It is suggested then the aminotransferase measurements are repeated to confirm elevated readings or in patients with NAFLD risk factors with normal results. **(Oh and Hustead, 2011).**

2. **Liver Biopsy**

Although liver biopsy is still considered the gold standard for NAFLD diagnosis, it presents with several limitations due to the invasiveness of the procedure and accuracy necessary to obtain the amount of tissue required for an accurate diagnosis. Further, mild discomfort or pain is experienced by 84% of patients following a liver biopsy with severe complications of extreme pain, major bleeding requiring a blood transfusion, infection, and death occurring in 0.3% of cases. In morbidly obese patients transjugular biopsy may be recommended for safety (complication rate of 1.3–6.5%), and a higher success rate compared to percutaneous biopsy **(Behrens and Ferral, 2012).**

3. **Non-invasive Diagnosis of NASH**

Non-invasive diagnostic models for NASH have not been validated in long-term studies; however, a variety of noninvasive markers are available to assist clinicians in their decision-making and diagnosis process. Clinical models predict NASH using anthropometric data and lab results routinely assessed in the clinic; however, they should be used to supplement clinical decision making for further screening. These models include HAIR, NASH Predictive Index and the NASH Clinical Scoring System for Morbid Obesity developed by associating clinical variables (features of the metabolic syndrome) with histologic features of NASH **(Alkhouri and McCullough, 2012).**

4. **NAFLD Biomarkers**

Several investigators are researching the use of biomarkers in distinguishing NAFLD by detecting serum markers of specific disease activity, including proteins associated with necrosis, fibrogenesis and the cytokines, adipokines, insulin resistance, and markers of apoptosis associated with the pathogenesis of NAFLD **(Golabi *et al*., 2015; Kamada *et al*., 2015).**

Thus far, however, none of the available biomarkers of NAFLD have been demonstrated to outperform clinical prediction models at detecting or ruling out NAFLD, making it difficult to justify the additional time and cost of biomarker testing **(Rossi *et al*., 2013).**

5. **Fibrosis Assessment**

A key element when assessing NAFLD patients is to determine the severity of fibrosis which has been found to be predictive of overall and disease specific mortality. Fibrosis scores can be estimated by non-invasive modalities, including clinical predictor models, biomarker panels, and elastography **(Fazel *et al*., 2016).**

Hepatocellular Carcinoma (HCC) in NAFLD

HCC is the six most common cancers worldwide; the third most common cause of cancer related death and has a globally rising incidence. Several studies have demonstrated an association between MetS, T2DM as well as obesity, with HCC, suggesting that NAFLD is playing a significant role in the rising incidence of HCC. The potential mechanisms relating MetS, obesity, diabetes, NAFLD, and HCC, particularly in the absence of cirrhosis, are probably related to the pathogenesis of the underlying disease rather than to fibrosis alone. A fertile soil for liver carcinogenesis include insulin resistance and hepatic steatosis promoting adipose tissue-derived inflammation, hormonal changes (adipokines), oxidative stress, lipopoxicity, and stimulation of insulin-like growth factor **(Dyson *et al*., 2014).**

Treatment

Lifestyle modification with a focus on healthy eating, weight loss when needed, and regular exercise remains the cornerstone of therapy in adults and children **(Africa *et al*., 2016).** When recommending healthy food choices, a Mediterranean diet has been shown to be a good alternative to a western diet **(Abenavoli *et al*., 2014).**

Bariatric surgery can be a good option in selected patients and a long term follow up study has been shown to reverse NASH and even substantial fibrosis in some **(Corey and Rinella, 2016; Lassailly *et al*., 2015).** However, surgery is possible in only a minority of patients and there is clearly a need for pharmacological therapy **(Ratziu, 2016; Musso *et al*., 2016).** Prior clinical trial data suggest that pioglitazone or vitamin E may be beneficial in non-diabetic NASH patients and the benefit of pioglitazone on reversing NASH and improving fibrosis was recently confirmed in diabetic patients **(Cusi *et al*., 2016).**

Future directions

Many of the current pharmacological approaches to treating NASH are focused on relatively downstream events of liver injury, inflammation and fibrogenesis. It may be advantageous to manipulate the upstream events leading to substrate overload such as central nervous system control of satiety mechanisms and energy efficiency ***(Camilleri, 2015; Cohen and Spiegelman, 2015 (Neuschwander-Tetri, 2017).***

Immunopathogenesis of NAFLD

A crucial role is played by inflammatory mediators, especially those deriving from adipose tissue and the gut, which are involved in the cascade of inflammation, fibrosis and eventually tumorigenesis. In this setting, endoplasmic reticulum stress, cytokines and adipokines as well as immunity are emerging drivers of the key features of NASH **(Tilg and Moschen, 2010).** Moreover, the liver itself displays immune properties, and can be viewed as an "immunological organ" **(Racanelli and Rehermann, 2006).** Many efforts have been undertaken to understand the role of the immune system in the pathogenesis of NASH, also in view of its potential therapeutic relevance. This review will focus on the disturbances of the cells constituting the innate and adaptive immune system in the liver and in the adipose tissue in NASH **(Vonghia *et al*., 2013).**

I- Innate Immunity

In recent years, the role of the innate immune response in NAFLD has been the focus of intense research. Activation and recruitment of immune cells in the liver by either local signals or signals derived from the adipose tissue or gut, related to changes in the microbial balance and/or bacterial translocation, may promote the inflammatory response, leading to cell injury and death, thus promoting NAFLD disease progression. In particular, the role of intracellular or surface-expressed pattern recognition receptors (PRRs), which are critical in recognizing cell damage and pathogen invasion, as relevant players in NAFLD/NASH is being unveiled **(Arrese *et al*., 2016).**

Damage-associated molecular patterns (DAMPS), pathogen-associated molecular patterns (PAMPs) and their receptors

Sterile inflammation occurs in the absence of pathogens or external antigens and is an important mechanism of liver injury in liver diseases **(Ganz and Szabo, 2013),** particularly in NASH, where it probably contributes to ongoing inflammation and disease progression **(Miura *et al*., 2013).** The acronym DAMPs refers to a set of intracellular molecules that are released or secreted upon the occurrence of cellular injury or death and seem to be key inducers of sterile inflammation **(Garcia-Martinez *et al*., 2015).**

In addition to DAMPs, PAMPs are also at play in determining liver injury in NAFLD/NASH. PAMPs refer to a number of bacterial products, including bacterial lipopolysaccharide (LPS), which derives from the cellular wall of gram-negative bacteria, and other molecules such as peptidoglycans, bacterial lipoprotein flagellins, bacterial RNA and DNA, and others, which can reach the liver upon disruption of the intestinal mucosal barrier and locally activate innate immune cells, triggering intracellular signaling cascades that amplify injury **(Arrese *et al*., 2016).**

DAMPs and PAMPs bind the PRRs, triggering a local inflammatory response and creating an injury amplification loop leading to organ damage. This is essentially mediated by the production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which, in addition to promoting inflammation, have important metabolic effects influencing insulin resistance and lipid metabolism **(Peverill *et al*., 2014).** Among PRRs, Toll-like receptors (TLRs) are the best characterized and comprise a family of cell surface and endocytic receptors expressed in most liver cells including hepatocytes, Kupffer cells (KCs), hepatic stellate cells (HSCs), biliary epithelial cells and sinusoidal endothelial cells, with each cell population exhibiting a different pattern of TLR expression. The most studied TLRs in the liver, and particularly in NASH, are TLR2, TLR4 and TLR9 **(Kesar and Odin, 2014).**

These receptors recognize specific invariant motifs present in pathogen molecules. While TLR2 recognizes peptidoglycans, TLR4 and TLR9 recognize bacterial LPS and DNA, respectively. The nucleotide oligomerization domain (NOD)-like receptors (NLRs) also belongs to the PRR family and can recognize DAMPs and PAMPs. Activation of NLRs promotes the assembly of inflammasome proteins, leading to cell death through the activation of caspase-1 and production of mature forms of IL-1 and IL-18 **(Szabo and Petrasek, 2015).**

The role of inflammasome activation in NAFLD/NASH has received significant attention in recent years. Work from one of the author's laboratories has demonstrated that NLRP3 inflammasome activation is associated with hepatocyte pyroptosis, an inflammasome- dependent cell death process, and that lack of this receptor attenuates inflammation and fibrosis in experimental NASH, underscoring the relevance of this pathway **(Wree *et al*., 2014).**

Cellular Players

Kupffer Cells (KCs)

KCs are the resident macrophages of the liver, located in the hepatic sinusoids, the portal tract and hepatic lymph nodes. This cell type derives from circulating monocytes and represents around 15 % of the liver cells, being the largest tissue-specific reservoir of macro-phages in the body **(Duarte *et al*., 2015).** In a healthy liver, the major immune function of KCs is to phagocytize pathogens or bacterial-derived products coming from the portal vein circulation, constituting the final barrier to prevent spreading of these products to the peripheral circulation. KCs also phagocytize cell debris of neighboring cells and present antigens to cytotoxic and

regulatory T cells. Activation of KCs plays a key role in NAFLD pathogenesis and progression, as demonstrated by studies showing that depletion of these cells attenuates insulin resistance, inflammatory development and even fibrosis **(Lanthier, 2015).**

Dendritic Cells

DCs predominate in physiological conditions, the mature and proinflammatory DC population prevails during liver injury DCs are tolerogenic immune cells located around the central veins and portal tracts that collectively represent a small fraction of non-parenchymal liver cells. DCs mainly originate in the bone marrow. DCs may act as antigen presenting cells as well as in apoptotic cell clearance and removal of necrotic debris, thus limiting sterile inflammation. The role of DCs in NASH is complex and controversial **(Tacke and Yoneyama, 2013).** Indeed, available information on the role of DCs in NASH is scarce, and the techniques used to assess its role in liver injury have shortcomings. It is thought that while immature and tolerogenic **(Lukacs-Kornek and Schuppan, 2013).**

Neutrophils

Neutrophil accumulation is one of the main features of NASH, and it is thought that this cell type critically contributes to hepatocellular damage in this setting as it can exacerbate the ongoing inflammatory state by contributing to macrophage recruitment and through interaction with antigen-presenting cells. The release of myeloperoxidase, a prooxidant neutrophil enzyme, seems to be a relevant mechanism that enhances macrophage cytotoxicity and promotes inflammation and fibrosis in experimental models **(Xu *et al*., 2014).**

Natural Killer (NK) and Natural Killer T Cells (NKT)

NK cells are lymphoid cells that play a role in linking the innate and adaptive immune responses within the liver. Interestingly, liver NK cells display different immune-phenotypical and functional characteristics than those of peripheral NK cells; this is thought to be related to cross-talk with other liver cells. NK cell functions are tightly regulated by the stimulation of diverse activating and inhibitory surface receptors. Different studies have shown that NK cells may be activated in NASH in connection with elevated levels of several NK cell-activating cytokines (e.g., IL-12, interferon-c and IL-18) and ligands **(Ganz and Szabo, 2013).**

Activation of Innate Immunity in NAFLD/NASH: The Gut Liver Axis and Adipose Tissue

The concept of the gut-liver axis refers to the existence of a physiological cross-talk between the liver and intestine consisting of a myriad of signals evoking relevant immunological and metabolic effects in the target organ. In fact, bile secretion from the liver has important consequences beyond fat digestion **(Kirpich *et al*., 2015).**

II- Adaptive Immunity

Adaptive immunity is characterized by antigenic specificity, diversity, immunologic memory, and self-non-self-recognition. This immune response is mediated by T and B lymphocytes and a variety of molecules that orchestrate cellular interactions **(Schildberg *et al*., 2015).**

T Lymphocytes

Although the total population of hepatic T lymphocytes (CD3+ lymphocytes) appears relatively stable in NASH, an imbalance of the different subtypes of CD3+ cells is observed in NASH. In particular an increased CD8+/CD4+ cell ratio has been described in the liver **(Henning *et al*., 2013).** At the level of the visceral adipose tissue, an increase of CD3+ cells have been described in humans and in mice and CD3 mRNA correlated with BMI. Accumulation of CD8+ and CD4+ cells was observed in adipose tissue inflammation and the latter cell subtype showed a TCR repertoire bias, suggestive of antigen-driven T cell activation, expansion and infiltration. CD4+ T cell transfer can negatively regulate weight gain, visceral adipose tissue mass, hyperglycaemia and cytokine increase (TNF-α and IL6) induced by HFD, predominantly through Th2 cells **(Cipolletta *et al*., 2011).**

T helper cells are a sub-group of lymphocytes that play an important role in the immune system and in particular in the adaptive immunity. Through cytokine release, they are able to drive the activation of the other immune cells as they are implicated in the B cell antibody class switching, in the activation of the cytotoxic T cells and in maximizing the bactericidal activity of phagocytes such as macrophages.

Th1 enhancement can induce, via INF-γ, the infiltration of M1 polarized macrophages in the adipose tissue of obese mice, accompanied by increased expression of TNF-α and MCP-1 **(Subramanian *et al*., 2015).**

Cytotoxic T cells (CD8+) rapidly increase in fad pads during HFD, prior to macrophage infiltration, and express a highly activated phenotype characterized by the release of pro-inflammatory mediators, which are implicated in the recruitment and activation of macrophages in the adipose tissue **(Kintscher *et al*., 2008).** Immunological or genetic depletion of CD8+ cells reduces macrophage infiltration, adipose tissue release of proinflammatory mediators (such as IL1, IL6 and MCP-1), and insulin resistance. These data highlight the role of CD8+ cells in initiating and propagating adipose tissue inflammation **(Nishimura *et al*., 2009).**

T Regulatory Cells

Treg cells derive from CD4+ Th0 cells in the presence of TGF-β and constitutively express the CD25 (IL2 receptor α chain). In addition, they express CD62L (glucocorticoid induced tumor necrosis factor receptor), CTLA4 (cytotoxic T lymphocyte associated protein) and FOXP3 (forkhead/winged helix transcription factor), the latter being crucial for their function **(Oo YH *et al*., 2010).**

Contrary to these data, immunohistochemical evaluation of liver biopsies from NAFLD/NASH patients showed an increase of FOXP3+ cells in NASH patients with a more advanced disease. FOXP3 positivity was distributed both in the lobule and in the portal tracts and higher FOXP3+/CD3+ quota positively correlated with the histologic severity of the disease. Therefore, it could be postulated that Tregs could be involved in the development of liver damage and that CD3+ cells could be diminished by T regs to decrease inflammation **(Soderberg *et al*., 2011).**

Furthermore, Tregs were investigated in the different sites of adipose tissue. FOXP3/CD4 expression was increased in the abdominal fat of 30-week-old mice fed a normal diet, FOXP3 being expressed in more than half of the CD4+ cells, in comparison with lymphoid or non-lymphoid tissues, such as liver and subcutaneous fat. Low T reg expression was observed in both the abdominal and subcutaneous fat deposits at birth and progressively accumulated in the abdominal adipose tissue but not in the subcutaneous tissue over time. Moreover Tregs were specifically reduced in the abdominal site in insulin-resistant models of obesity, with a mechanism related, at least in part, to the suppressive ability of leptin on Treg proliferation **(Naylor and Petri, 2016).**

This dichotomy between subcutaneous and abdominal fat is in line with the well-known association of the latter with insulin resistance **(Tanisawa *et al*., 2017).**

In type 2 diabetes the ratio between Tregs and Th17 was decreased. Tregs appeared to be more prone to cell death and their reduction was more pronounced in patients with microvascular rather than with macrovascular complications **(Zeng *et al*., 2012).**

The regulation of the T reg/Th17 axis in these patients could be, at least in part, due to the action of IL6 and to its capability to interact with these cells either by binding the IL6 receptor (IL6-R) on different cell types or through a trans-signaling mechanism that involves the soluble sIL6-R **(Ryba-Stanislawowska *et al*., 2013).**

Th17

Th17 cells are a subtype of T helper cells that are characterized by the secretion IL17 and which differentiation is specifically induced by the transcription nuclear factor retinoic acid receptor-related orphan receptor (ROR)-γt. Th17 are generated in the presence of TGF-β and IL6 and exert pro-inflammatory functions **(Zhao *et al*., 2010).**

This subset of cells functionally opposes T reg mediated response and has reciprocal developmental pathways, having antithetical effects in the immune response. The Treg transcription factor FOXP3 has a direct inhibitory effect on the differentiation of Th17, binding the Th17 specific transcription factor ROR-γt. Moreover, Tregs may convert to Th17 in the context of pro-inflammatory stimuli, losing their suppressive function **(Abdulahad *et al*., 2011).**

Moreover, IL17 and IL23, which are implicated in the Th17 pathway, appeared to be increased in obese patients and positively correlated with elevated levels of leptin, a pro-inflammatory and anorexigenic adipokine **(Sumarac-Dumanovic *et al*., 2009).**

In line with these findings, a recent study showed that leptin deficient (ob/ob) or leptin-receptor deficient (db/db) mice displayed lower levels of IL17, in comparison to wild type (WT) mice, indicating an impairment of the IL17 pathway in conditions of leptin downregulation. Moreover, IL17 pathway resulted in being enhanced, in a dose dependent manner, by leptin. Indeed, increasing doses of leptin were able to raise the number of splenic Th17 as well as the production of IL17 and of the Th17-specific transcription nuclear factor ROR-γt in ob/ob leptin deficient mice **(Yu Y *et al*., 2013).**

Contrary to these findings, Th17 were reduced in mice fed HFD at the level of visceral adipose tissue and IL17 acted as a negative regulator of adipogenesis and glucose metabolism in mice, and delayed the development of obesity **(Zuniga *et al*., 2010).**

The IL17 pathway appears to be implicated also in the pathogenesis of liver fibrosis, which plays a key role in the progression of liver disease. In hepatotoxic and cholestatic mouse models, liver injury enhanced IL17 signaling, as revealed by the increase of IL17 and its receptor and by the consequent activation of inflammatory and liver resident cells. In fact, IL17 induced an increased production of IL6, IL1, TNF-α and TGF-β1 by inflammatory cells as well as an increased deposition of collagen type 1 by HSC, via signal transducer and activator of transcription 3 (STAT3) activation **(Meng *et al*., 2012).**

B Lymphocytes

B lymphocytes constitute around 6% of intrahepatic cells. Moreover, they rapidly increase in serum and adipose tissue of mice fed HFD, and seem to be implicated in insulin resistance. Namely, B-cell-deficient mice fed HFD show a lower insulin resistance than controls. Accordingly, adoptive transfer of B cells or IgG isolated from mice fed HFD into B-cell-deficient mice determines the onset of insulin resistance. Moreover, patients with insulin

resistance display a distinct IgG profile compared to subjects without insulin resistance **(Winer *et al*., 2011).**

In addition, an increase of the serum level of B-cell-activating factor (BAFF) has been described in human NASH. Preclinical studies showed that BAFF receptor deficient mice display an improvement in HFD-induced obesity and insulin resistance accompanied by a reduction of B cells, serum IgG levels and visceral adipose tissue inflammation. Moreover, BAFF was found to be able to downregulate steatogenesis genes and to enhance steatosis in hepatocytes through BAFF-R, indicating a protective role of BAFF in hepatic steatosis by regulating lipid metabolism in the liver **(Kawasaki *et al*., 2013).** B2 cells could also be altered in obesity as suggested by the observation of a lower antibody response to tetanus toxin immunization in overweight children and mice **(Eliakim *et al*., 2006).**

III- Adipokines and Soluble Mediators

A relevant role in the frame of the "multiple parallel hits hypothesis" is played by the balance of adipose tissue derived mediators, such as adiponectin and leptin. Moreover, the role of ghrelin, visfatin and resistin has been investigated **(Gonciarz *et al*., 2013).**

IV- Microbiota

The microbiota consists of complex communities of microorganisms, which populate the skin and mucosal tissues throughout the body, forming a nourished ecosystem within its host. Thou-sands of years of microbial and immune co-evolution have led to a harmonious co-existence between the host and its colonizing microbes shaping the repertoire of both the host's immune system and microbiota reciprocally **(Maynard *et al*., 2012).**

V- MicroRNAs

MicroRNAs (miRNAs) are highly conserved, small, 18–25 nucleotide, non-coding RNAs that regulate gene expression at the post-transcriptional level. In most cases, miRNAs bind to the 30 un-translated region (UTR) of the target mRNA repressing the translation by destabilizing mRNA and/or silencing translation. However, in some instances they can interact with their targets in a non-30 UTR dependent manner and cause the up-regulation of their targets **(Bala *et al*., 2011).**

Therapeutic Implications of immunomodulator

Currently there is no approved pharmacological treatment available for NASH. The emerging role of disturbances of the immune system in the pathogenetic mechanisms of NASH opens perspectives for new potential therapeutic options through immuno-regulation **(Von Boehmer, H.; Daniel, 2013).**

Preliminary trials have used the anti-CD3 moAb, which is able to prevent induction and progression of inflammatory and autoimmune diseases. Preclinical studies showed the efficacy of anti-CD3 moAb or of its F (ab1)2 in controlling insulin resistance in leptin deficient ob/ob and wild type mice. A 5-day short term treatment course appeared to be able to restore T regs in the visceral adipose tissue and to improve glucose tolerance and insulin sensitivity, despite continuation of HFD **(Winer *et al*., 2009).**

Another approach consisted of oral administration of anti-CD3 moAb paired with β-glucosylceramide (GC). Oral anti-CD3 antibody is rapidly taken up by the gut-associated lymphoid tissue (GALT) and induces CD4+CD25−latency-associated peptide (LAP)-positive Tregs, which act in a TGF-β-dependent manner. β-GC is an intermediate in the metabolic pathway of glycosphingolipids, which is able to interact with CD1d, a ligand of NTK. Treatment resulted in a decrease in pancreatic islet cell hyperplasia, fat accumulation in the liver and inflammation in adipose tissue, and was accompanied by lower blood glucose and liver enzymes. In addition, an increase of CD11b+F4/80+ macrophages and TNF-α in the adipose tissue was observed **(Ilan *et al*., 2010).**

Probiotics in treatment NFALD

NASH Data in animal models have indicated the beneficial effects of prebiotics, probiotics and symbiotic preparations in the management of NAFLD. In this regard, **Li Z *et al*. (2003)** found that 4 weeks of treatment with VSL#3, a cocktail containing 8 live bacterial strains consisting of: Lactobacillus, Bifidobacterium species and a streptococcal strain, was associated with improved NAFLD histology and a reduction in hepatic total fatty acid content as well as reductions in serum ALT levels in ob/ob mice fed with a high fat diet. These effects were accompanied by reductions in activity of JNK and decreases in the DNA-binding activity of NF-κB.

Summary and Conclusion

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease worldwide. The US guidelines for NAFLD management define NAFLD as a) steatosis with ≥5% fat infiltration in imaging or histology and b) no alcohol, drug or viral induced steatosis. Furthermore, diagnosis of NAFLD requires exclusion of other liver diseases, such as alcoholic liver disease, viral hepatitis, and Wilson's disease.

Over the last decade, it has been shown that the clinical burden of NAFLD is not only confined to liver-related morbidity and mortality, but there is now growing evidence that NAFLD is a multisystem disease, affecting extra-hepatic organs and regulatory pathways. For example, NAFLD increases risk of type 2 diabetes mellitus (T2DM), cardiovascular (CVD) and cardiac diseases, and chronic kidney disease (CKD).

Although the primary liver pathology in NAFLD affects hepatic structure and function to cause morbidity and mortality from cirrhosis, liver failure and hepatocellular carcinoma, the majority of deaths among NAFLD patients are attributable to CVD.

The pathogenesis of NAFLD is complex and implicates cross-talk between different metabolically active sites, such as liver and adipose tissue. Obesity is considered a chronic low-grade inflammatory state and the liver has been recognized as being an "immunological organ". The complex role of the immune system in the pathogenesis of NAFLD is currently raising great interest, also in view of the possible therapeutic potential of immunotherapy in NAFLD.

From the immunological standpoint of view, the role of immune system disturbances in the NAFLD multifactorial mechanisms is increasingly being recognized.

Both the innate and adaptive immune systems are involved, and they are disturbed at different levels. They display not only tissue specific modifications (e.g., liver and adipose tissue) but also, within the same tissue, location-specific (e.g., visceral and subcutaneous adipose tissue) discrepancies.

Of note is the imbalance of the T reg/Th17 axis, which could become a target of novel therapies addressed either to enhance the Treg compartment or to suppress the Th17 pathway (e.g., inhibiting ROR-γt). Adipokines contribute to the metabolic and inflammatory features of the disease. Leptin, in particular, is involved in constituting a loop between nutrition, metabolism and the immune system.

Research is continued for the clarification of the immune system dysfunction associated with non-alcoholic fatty liver diseases and to decide if these changes are consequences or plays a role in the pathogenesis of the disease.

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