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**Cross talk of the immune system in the adipose tissue and the liver in non-alcoholic steatohepatitis: Pathology and beyond**

Vonghia L *et al*. Immune system and non-alcoholic steatohepatitis

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**Abstract**

Non-alcoholic steatohepatitis (NASH) is considered to be the hepatic manifestation of the metabolic syndrome, thus has a tight correlation with systemic metabolic impairment. The complex mechanisms underlying the pathogenesis of NASH involve different organs and systems that cross talk together contributing to the onset of NASH. A crucial role is played by inflammatory mediators, especially those deriving from the adipose tissue and the liver, which are involved in the cascade of inflammation, fibrosis and eventually tumorigenesis. In this setting cytokines and adipokines as well as immunity are emerging drivers of the key features of NASH. The immune system participates in this process with disturbances of the cells constituting both the innate and the adaptive immune systems that have been reported in different organs, such as in the liver and in the adipose tissue, in clinical and preclinical studies. The role of the immune system in NASH is increasingly studied, not only because of its contribution to the pathogenetic mechanisms of NASH but also because of the new potential therapeutic options it offers in this setting. Indeed, novel treatments acting on the immune system could offer new options in the management of NASH and the correlated clinical consequences.

**Key words:** Non-alcoholic steatohepatitis; Immune system; Adipokines; Inflammation; Fibrosis

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**Core tip:** Non-alcoholic steatohepatitis (NASH) is considered to be the hepatic manifestation of the metabolic syndrome, thus has a tight correlation with systemic metabolic impairment. The complex mechanisms underlying the pathogenesis of NASH involve different organs, including liver, adipose tissue and immune system, which cross talk together contributing to the onset of NASH. Increasing interest has been aroused by the role of the immune system in NASH, not only because of its contribution to the pathogenetic mechanisms of NASH but also considering the new potential therapeutic options in this setting.

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**INTRODUCTION**

The increasing burden of non-alcoholic fatty liver disease (NAFLD) is a major health concern. The NAFLD worldwide prevalence shows an upward trend over time and has reached “pandemic” proportions. In the general population it is estimated to be 20%-30% in Western countries and 5%-18% in Asia and is associated with an increased prevalence of obesity, insulin resistance, metabolic syndrome and diabetes, which are often paired to NAFLD[1]. Indeed in at risk patients, such as patients with diabetes mellitus, the prevalence of NAFLD increases up to 40%-70%[2]. In addition, NAFLD can run a unfavourable course, given the possible evolution to cirrhosis and hepatocellular carcinoma and can constitute an indication for liver transplantation[3].

NAFLD and more specifically non-alcoholic steatohepatitis (NASH) are closely related to metabolic impairment, such as visceral adiposity, hyperinsulinaemia or diabetes, dyslipidaemia and arterial hypertension, which define the metabolic syndrome. NAFLD and NASH are considered the hepatic manifestation of the metabolic syndrome[4]. Moreover patients with NAFLD, and a fortiori NASH, are at higher risk of developing diabetes mellitus and are at increased risk of morbidity and mortality related to cardiovascular diseases[3,5].

These considerations arise the need of understanding the complex mechanisms underlying the onset of NASH. At the basis of a wide clinical spectrum of NAFLD that includes metabolic impairment at different levels, there is a complex interaction between different organs at the pathogenetic level. This is conceptualized in the “multiple parallel hit hypothesis”[6] and has been substantiated by further research. The liver damage, driven by insulin-resistance, iron accumulation, oxidative stress and hepatocyte death, can be triggered by an imbalance in anti- and pro-inflammatory factors originating from the liver itself or from extrahepatic sites that cross talk with the liver, particularly the adipose tissue and the gut[7]. Another key player in the pathogenesis of NASH is the immune system, including both the innate[8] and the adaptive[9] immune cells[10]. The specific role of the different cell-subsets and the reciprocal role of pro- and anti-inflammatory pathways, however, have not yet been fully clarified and is object of interest in NASH research. Understanding the reciprocal role of these cells in NAFLD should help identifying possible targets for treatment, as nowadays there is no pharmacological treatment licensed for NAFLD[4].

**LIVER, ADIPOSE TISSUE AND THE IMMUNE SYSTEM**

NAFLD and NASH are associated with the presence of low-grade inflammation. Numerous studies have demonstrated that both the innate and the adaptive immune system play an important role in the pathogenesis of NAFLD/NASH (for review see[10]) and, moreover, that the organ-specific immunity is involved in the onset and progression of this disease.

When considering the whole population of T lymphocytes (CD3+ leucocytes) in the liver, it appears relatively stable in NASH. A variation of the various subtypes of CD3+ cells, however, has been described in NASH, namely a relative increase of the hepatic CD8+ cells in comparison with the CD4+ cells (hence a higher CD8+/CD4+ ratio)[11]. Among the CD4+ cells, an imbalance between the T helper (Th) 1 and Th2 profile towards the pro-inflammatory Th1 has also been described[12]. Moreover a liver specific and reversible depletion of the regulatory T-cells (Tregs) was observed under high fat diet (HFD) in an animal model[13]. The Treg decrease in NASH can in part be explained by dendritic cells (DC) induced down-regulation. In vitro studies indeed demonstrated that intrahepatic DC are able to blunt the CD25+FOXP3+ Treg phenotype within the CD4+ cells[11].

Opposite to these findings, in liver biopsies from a group of NAFLD patients, including NASH patients, the forkhead/winged helix transcription factor (FOXP3) positive cells (Tregs)[14] were more expressed in NASH patients with a more severe disease[15] in comparison with no-NASH patients, hence showing a Treg proliferation with the progression of the disease.

The Th17 pathway is another key player in liver disease, including NAFLD and NASH. In preclinical and clinical studies an increase of the Th17 cells was described together with an up-regulation of the Th17-related genes. Moreover IL17 appeared to be crucial in the induction of liver injury in a HFD context and is implicated in metabolic damage by interfering with the insulin signalling pathway[16]. A stimulation of the Th17 occurs, at least in part, *via* interaction between liver and adipose tissue. Indeed, leptin, an anorexigenig and pro-inflammatory adipokine which is increased in obesity due to a mechanism of leptin resistance[17], is able to increase the number of Th17 and the gene expression of the Th17-specific transcription nuclear factor RAR-related orphan receptor (ROR)γt and to stimulate the IL17 production[18]. In addition, the IL17 pathway is implicated in the onset of liver fibrosis: liver injury induces IL17 signalling, which in turn stimulates collagen deposition from the hepatic stellate cells (HSC) and hence the onset of fibrosis[19]. An impairment of the balance between Tregs and Th17 is hence potentially of relevance for the onset and development of NASH, which opens perspectives for new treatment.

Natural killer T (NKT) cells are reduced in hepatic steatosis[20-22], but are increased in hepatic fibrosis in the context of NASH[20,23]. Indeed, human liver biopsies with advanced fibrosis showed increased levels of osteopontin (OPN) and hedgehog (Hh), which are secreted by NKT, in comparison with early stages of fibrosis[24].

The resident macrophages in the liver, the Kupffer cells (KC), are sensitive to gut-derived endotoxin and modulate the activation of different cells in the liver, such as dendritic cells (DCs), T lymphocytes and neutrophils[25]. They are actively implicated in the development and progression of NASH by the secretion of tumour necrosis factor (TNF)-α, which plays an important role in the early phase of the disease, and of IL6, which is important in the liver disease evolution and in de onset of insulin resistance[7].

DCs in NASH are enrolled in the early phases. They display a decreased plasmocytoid and lymphoid fraction and an increased myeloid fraction and produce higher levels of pro-inflammatory cytokines and to mediate an allogenic T cell proliferation, an antigen-restricted CD4+ T cell stimulation and a Treg down-regulation[11].

The adipose tissue is another key organ in the pathogenesis of NASH and the associated metabolic impairment. Moreover the NASH-related immune system impairment involves also the immune cells infiltrating this organ.

Considering the T lymphocytes, CD8+ and CD4+ are enriched in the adipose tissue[11]. Moreover there is a shift towards the pro-inflammatory Th1 cytokines in comparison with the anti-inflammatory Th2 ones, particularly in the visceral adipose tissue[12]. Interestingly, the Th1 stimulation, *via* INFγ, induces the infiltration of the adipose tissue by other pro-inflammatory cells, such as the M1-polarized macrophages[26].

The abdominal adipose tissue (but not the subcutaneous adipose tissue) is a preferential source of Tregs in mice fed a normal diet with a time-dependent kinetic. In insulin-resistant models of obesity Tregs are specifically reduced in the abdominal site[12,27], which can be explained, at least in part, by the suppression of Treg proliferation by leptin[28]. Moreover in obese patients FOXP3 RNA was expressed at a higher level in the subcutaneous adipose tissue and a negative correlation between BMI and the FOXP3-to-CD3 ratio in omental versus subcutaneous fat was reported in these patients[27]. In leptin deficient obese mice Treg depletion leads to increased fasting blood glucose level, impaired insulin sensitivity and renal impairment, while Treg adoptive transfer improves insulin resistance[29]. In addition, in type 2 diabetes a deregulation of the balance between Tregs and Th17 occurs: there is a decrease of the Tregs/Th17 ratio and Tregs appear to be more prone to cell death[30]. Opposite to these findings, other studies suggested a potential beneficial effect of the IL17 in blunting the phenotypic and metabolic characteristics correlated to obesity. Preclinical studies showed a reduction of the Th17 in the visceral adipose tissue of mice fed a HFD[12] and demonstrated the role of IL17 as a negative regulator of adipogenesis and glucose metabolism in mice, delaying the onset of obesity[31]. In these experiments, IL-17 deficiency enhanced diet-induced obesity, early adipose tissue accumulation and altered glucose homeostasis. In addition IL-17 acted on preadipocytes and adipocytes to inhibit adipogenesis and moderate lipid and glucose uptake[31].

A depletion of invariant Natural Killer T cells (iNTK) has been reported in obesity, in correlation with pro-inflammatory macrophage infiltration. Indeed, iNKT-depleted NASH animal models show larger adipocytes while iNKT adoptive transfer decreases fat accumulation, leptin levels and insulin sensitivity[32].

In adipose tissue, an infiltration of DC has been shown in preclinical and clinical studies. In humans, the subcutaneous adipose tissue-derived DC have been described to correlate with metabolic impairment (high Body Mass Index (BMI) and insulin resistance) and with increased Th17[33].

Considering the B lymphocytes, they contribute to the onset of insulin resistance. Mice fed a HFD display and increase of B lymphocytes in serum and adipose tissue, while when feeding B-cell-deficient mice a HFD lower insulin resistance is determined. Accordingly, adoptive transfer of B cells or IgG isolated from mice fed a HFD into B-cell-deficient mice induces insulin resistance. In addition, insulin-resistant patients have a distinct IgG profile compared to patients without it[34].

Macrophages derive from circulating monocytes and play a crucial role in the adipose tissue. They can activate as the “classically activated” pro-inflammatory M1 or as the “alternatively activated” anti-inflammatory M2 states. In obesity animal models pro-inflammatory M1 polarized macrophages infiltrate the adipose tissue[32] and create the characteristic “crown like” structures around necrotic adipocytes[32].

The obesity-related switch from the M2 to the M1 polarization is driven by a C-C chemokine receptor 2 (CCR2)-dependent monocyte recruitment[35]. CCR2 is therefore a potential target of therapy. Indeed, blunting macrophage accumulation, also *via* monocyte chemoattractant protein 1/chemokine (C-C motif) ligand 2 (MCP-1/CCL2) inhibition, induces an improvement of inflammation activity, insulin resistance and liver fibrosis[7].

These data summarize the multiple immune cell subtypes involved in the onset of NAFLD and NASH, which draw complex pathways and offer various possible targets to interfere with the onset of NASH.

A relevant role in the pathogenesis of NASH is played by adipose tissue-derived mediators, such as adiponectin and leptin [6], and other molecules such as ghrelin, visfatin and resistin[36,37].

Adiponectin and leptin are produced mainly by the adipose tissue. The former acts as an insulin sensitizing and an anti-inflammatory mediator. Hypoadiponectinemia has been found to be associated with the metabolic syndrome and its components, including NASH[38]. The latter, under physiological conditions, has anorexigenic effects decreasing appetite and increasing energy expenditure, while in obese patients hyperleptinemia associated to leptin resistance has been described [17]. Moreover leptin has pro-inflammatory and profibrogenic properties that play a role in liver disease, including NASH[37,39,40].

Adiponectin exerts its anti-inflammatory function inhibiting the pro-inflammatory cytokines (TNF-α) and stimulating the anti-inflammatory cytokines (IL10 secreted by KC)[41] and *via* direct suppression of the macrophage function[42]. Adiponectin attenuates also oxidative stress and fibrogenesis, the latter through suppression of the activated HSC function[38].

Leptin is able to affect the production of acute-phase-reactants, such as IL1 and TNF-α, to alterate the Th1/Th2/Tregs balance promoting a Th1 differentiation and a Treg down-regulation[40]. Hyperleptinemia is a condition correlated with obesity and can favour pro-inflammatory mechanisms. Namely it can induce a proliferation of Th1 cells in the adipose tissue, of CD8+ T cells, macrophages and mast cells and stimulates pro-inflammatory cytokines (as TNF-α, IL6 and IL12). Moreover they induce a down-regulation of the Treg in the adipose tissue, as previously described[40].

Ghrelin is a gut peptide that is involved in regulation of food intake and energy balance. Ghrelin has been reported to have protective effects on the liver and reduced levels of this hormone have been found in NAFLD patients[43].

Resistin, which is produced by adipose tissue and macrophages, is involved in insulin resistance, has pro-inflammatory (*via* stimulation of the secretion of TNF-α and IL12 by macrophages and *via* regulation of IL6 and IL1β production) and profibrogenic (acting on the HSC)[36]. Resistin has been correlated with the progression of liver damage in NAFLD and with the onset of NASH[44].

NASH patients show lower adiponectin, higher leptin and resisitin and unaltered ghrelin levels in comparison with control subjects. In these patients antioxidant treatment can induce an reversal of the hypoleptinemia and hypoadiponectinemia and is able to arise the ghrelin levels[37].

Visfatin is an insulin mimicking adipokine. It is able to induce lL6 secretion from CD4+ T cells[45]. The specific contribution in NASH, however, has not been fully clarified.

**TREATMENT PERSPECTIVES**

Currently there is no approved pharmacological treatment available for NASH. Among the treatments used in the pharmacotherapy of NASH, some agents have failed to give a satisfactory improvement of NASH, such as metformin, statins and ursodeoxycholic acid. Vitamin E and thiazolidinediones have shown beneficial effects on liver histology in randomized control trials and can hence be used for the treatment of NASH, but are not approved for this indication[4]. Furthermore, there is some concern about the potential side effects associated with these drugs, which should hence be prescribed with caution[46]. Pioglitazone finds a possible indication in older patients with aggressive NASH and Vitamin E can be used in non-diabetic pre-cirrhotic adults[4]. Of note pioglitazone is also able to increase adiponectin levels[47] (Table 1).

Very recent preclinical data show the ability of the adiponectin receptor agonist AdipoRon to significantly ameliorate glucose metabolism and serum lipid levels. In the liver the AdipoRon reduces triglyceride content, oxidative stress, and inflammatory cytokine expression, suggesting its potential role in the treatment of NASH[48].

Another treatment approach exploits the possibility to interfere with the immune system, which is actively involved in the physiopathology of NASH, through immune-regulation[49].

Some data are available regarding the anti-CD3 monoclonal Antibody (moAb), which prevents the onset and the evolution of inflammatory and autoimmune diseases.

The anti-CD3 moAb or its Fragment anti-binding F(ab1)2 have been shown to be effective in ameliorating insulin resistance in leptin deficient *ob/ob* mice where they restored Tregs in the visceral adipose tissue and improved glucose tolerance and insulin sensitivity[12].

The anti-CD3 moAb can be also administered in combination with β-glucosylceramide (GC), which is able to mediate the interaction with other immune cells such as NTK. Oral anti-CD3 antibody is rapidly absorbed 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. Treatment of *ob/ob* mice resulted in a better metabolic control and an improvement of the liver damage. A decrease in pancreatic islet cell hyperplasia, fat accumulation in the liver and inflammation in adipose tissue, accompanied by lower blood glucose and liver enzymes were observed[50].

The systemic administration of anti-CD3 moAb, however, can be hampered by serious side effects such as the cytokine release syndrome, a “cytokine storm” released as a consequence of generalized T cell activation, or the antiglobulin response[51]. To minimize the side effects of the systemic administration of the anti-CD3 moAb and to maximize its local effects, anti-CD3 can be orally administered. A single-blind randomized placebo-controlled phase 2a study showed the safety of oral anti-CD3 moAb in 36 NASH patients with impaired glucose control up to type-2 diabetes. Oral anti-CD3 moAb showed safety and were able to improve liver damage and glucose metabolism. This effect was coupled with a persistent Treg level increase[51].

The Treg-induction can be either antigen-specific or antigen nonspecific. The induction of antigen-specific Tregs has the potential advantage of inducing a specific immune modulation and of reduced side effects. This is, however, not achievable in conditions such as type 2 diabetes or NASH where there are to date no well-defined target antigens. In these conditions the induction of antigen non-specific Tregs by anti-CD3 may be a valid option. Further research will investigate the possibility of developing a combination of mucosal anti-CD3 with a given antigen[51].

Moreover Tregs are an important possible target for immunotherapy. Different therapeutic approaches have been used to modulate these cells. The Imm124-E, an anti- lipopolysaccharide (LPS) hyperimmune bovine colostrum, has been tested, in an open label trial, in patients with biopsy-proven NASH and insulin resistance. Imm124-E was safe and effective in ameliorating the glucose metabolism parameters, the lipid profile and the liver injury. The improvement of the clinical parameters was paired with a Treg enhancement[52].

A redistribution of the Tregs, paired to an increase in NKTs, was reached in leptin deficient *ob/ob* and HFD mice treated with DT56a, a molecule contained in soybean able to activate estrogen receptors and to improve glucose homeostasis, the lipid profile and the liver enzymes[53].

Adoptive cell transfer refers to the transfer of immune cells into a recipient host aiming at transferring the immunological functionality into the host. In particular the Treg cell transfer is able to preserve and restore tolerance to self-antigens and alloantigens. The benefits of this treatment are the potential for antigen specificity, the lack of general immunosuppression and the long-lasting regulation[54]. Treg expansion in obese mice tempers TNF-α-related inflammation[13] but it is not able to restore metabolic function in obesity[27].

Cellular therapy has also been tested with other cell subtypes. The CD4+ Tcell transfer into obese mice reversed weight gain and insulin resistance[12]; iNKT transfer decreased body fat, triglyceride levels, leptin levels, hepatic steatosis and insulin sensitivity[32].

Although cellular therapy shows positive preliminary result and constitutes a conceptually potentially effective therapy, these treatments raise feasibility concerns in the clinical setting[55] and need further development and evaluation.

A further possible therapeutic approach involves the ROR pathway. RORα and RORγ are transcription factors implicated in the control of lipid and glucose metabolism, besides various immune functions. The absence of ROR*α* protects against diet-induced obesity, adipose tissue-associated inflammation, liver injury (namely steatosis), and insulin resistance[56]; RORγ deficiency also protects against diet-induced insulin resistance[57]. Therefore, ROR antagonists may provide a novel therapeutic target in the management of various aspect of the metabolic syndrome.

 Recently RORγt ligands have been studied in autoimmune diseases. They blunt the production of IL17 from the stimulated Th17 cells, by counteracting nuclear receptor specific for Th17 RORγt. These compounds constitute a promising strategy in the therapy of NASH, considering the central role of the Th17 pathway in the induction and progression of both the liver damage and the metabolic impairment.

Antioxidants constitute another potential therapy in NAFLD. Polyphenols, such as resveratrol contained in grapes and wine, are molecules of interest. Indeed resveratrol is able to improve insulin sensitivity and to modulate mitochondrial energetics[58]. Moreover resveratrol has been shown to be effective in ameliorating liver enzymes, insulin resistance and glucose and lipid metabolism in patients with NAFLD. Furthermore it induced a reduction of pro-inflammatory and profibrogenic cytokine levels (namely TNFα, cytokeratin 18 fragment, and fibroblast growth factor (FGF)) and an elevation of adiponectin levels[59].

Another potential target is the fibrosis pathway. The Chemokine receptors type 2 and 5 (CCR2- CC5) are expressed by cells involved in fibrogenesis, such as monocytes, macrophages, Kupffer cells and hepatic stellate cells. Preclinical studies in NASH and liver fibrosis animal models showed that a dual CCR2 and CCR5 inhibitor, Cenicriviroc (CVC), has an antifibrogenic effect[60]. A clinical study conducted in HIV patients has shown that CVC is able to improve lipid metabolism (decreasing the total cholesterol, LDL and triglycerides levels and increasing the HDL levels) and the fibrosis scores APRI[61] and FIB-4[62,63]. This, together with the preclinical data, makes CVC a good candidate for the treatment of NASH.

Very recently a new potential target of treatment for NAFLD has been identified. The Vascular adhesion protein-1 (VAP-1) is an amino-oxidase constitutively expressed on human hepatic endothelium that promotes lymphocyte recruitment in the liver. This molecule is increased in various models of liver disease, including NAFLD, and is implicated in both inflammation and fibrosis. In a NAFLD preclinical model VAP-1 inhibition leads to less leucocyte recruitment in the liver and, more specifically, a reduction of the CD4+ T lymphocytes and of the natural killer (NK) T lymphocytes. This, together with its antifibrogenic effect, makes the VAP-1 inhibition a potential therapeutic target[64].

Further research is, however, urgently needed to unravel the exact pathogenetic mechanisms of NAFLD/NASH, also aiming at discovering new effective therapeutic options, given the increasing burden of this disease and its potential evolutive course.

**REFERENCES**

1 **Masarone M**, Federico A, Abenavoli L, Loguercio C, Persico M. Non alcoholic fatty liver: epidemiology and natural history. *Rev Recent Clin Trials* 2014; **9**: 126-133 [PMID: 25514916 DOI: 10.2174/1574887109666141216111143]

2 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]

3 **Anstee QM**, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 330-344 [PMID: 23507799 DOI: 10.1038/nrgastro.2013.41]

4 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

5 **Francque S**, Laleman W, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, Van Dyck C, D'Hondt M, Ramon A, Vermeulen W, De Winter B, Van Marck E, Van Marck V, Pelckmans P, Michielsen P. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. *Lab Invest* 2012; **92**: 1428-1439 [PMID: 22890552 DOI: 10.1038/labinvest.2012.103]

6 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]

7 **Tran A**, Gual P. Non-alcoholic steatohepatitis in morbidly obese patients. *Clin Res Hepatol Gastroenterol* 2013; **37**: 17-29 [PMID: 23347840 DOI: 10.1016/j.clinre.2012.07.005]

8 **Maher JJ**, Leon P, Ryan JC. Beyond insulin resistance: Innate immunity in nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 670-678 [PMID: 18666225 DOI: 10.1002/hep.22399]

9 **Kaminski DA**, Randall TD. Adaptive immunity and adipose tissue biology. *Trends Immunol* 2010; **31**: 384-390 [PMID: 20817556 DOI: 10.1016/j.it.2010.08.001]

10 **Vonghia L**, Michielsen P, Francque S. Immunological mechanisms in the pathophysiology of non-alcoholic steatohepatitis. *Int J Mol Sci* 2013; **14**: 19867-19890 [PMID: 24084730 DOI: 10.3390/ijms141019867]

11 **Henning JR**, Graffeo CS, Rehman A, Fallon NC, Zambirinis CP, Ochi A, Barilla R, Jamal M, Deutsch M, Greco S, Ego-Osuala M, Bin-Saeed U, Rao RS, Badar S, Quesada JP, Acehan D, Miller G. Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice. *Hepatology* 2013; **58**: 589-602 [PMID: 23322710 DOI: 10.1002/hep.26267]

12 **Winer S**, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 2009; **15**: 921-929 [PMID: 19633657 DOI: 10.1038/nm.2001]

13 **Ma X**, Hua J, Mohamood AR, Hamad AR, Ravi R, Li Z. A high-fat diet and regulatory T cells influence susceptibility to endotoxin-induced liver injury. *Hepatology* 2007; **46**: 1519-1529 [PMID: 17661402 DOI: 10.1002/hep.21823]

14 **Oo YH**, Hubscher SG, Adams DH. Autoimmune hepatitis: new paradigms in the pathogenesis, diagnosis, and management. *Hepatol Int* 2010; **4**: 475-493 [PMID: 20827405 DOI: 10.1007/s12072-010-9183-5]

15 **Söderberg C**, Marmur J, Eckes K, Glaumann H, Sällberg M, Frelin L, Rosenberg P, Stål P, Hultcrantz R. Microvesicular fat, inter cellular adhesion molecule-1 and regulatory T-lymphocytes are of importance for the inflammatory process in livers with non-alcoholic steatohepatitis. *APMIS* 2011; **119**: 412-420 [PMID: 21635548 DOI: 10.1111/j.1600-0463.2011.02746.x]

16 **Tang Y**, Bian Z, Zhao L, Liu Y, Liang S, Wang Q, Han X, Peng Y, Chen X, Shen L, Qiu D, Li Z, Ma X. Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *Clin Exp Immunol* 2011; **166**: 281-290 [PMID: 21985374 DOI: 10.1111/j.1365-2249.2011.04471.x]

17 **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. The potential adverse role of leptin resistance in nonalcoholic fatty liver disease: a hypothesis based on critical review of the literature. *J Clin Gastroenterol* 2011; **45**: 50-54 [PMID: 20717042 DOI: 10.1097/MCG.0b013e3181ec5c66]

18 **Yu Y**, Liu Y, Shi FD, Zou H, Matarese G, La Cava A. Cutting edge: Leptin-induced RORγt expression in CD4+ T cells promotes Th17 responses in systemic lupus erythematosus. *J Immunol* 2013; **190**: 3054-3058 [PMID: 23447682 DOI: 10.4049/jimmunol.1203275]

19 **Meng F**, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Osterreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology* 2012; **143**: 765-766.e1-3 [PMID: 22687286 DOI: 10.1053/j.gastro.2012.05.049]

20 **Tajiri K**, Shimizu Y, Tsuneyama K, Sugiyama T. Role of liver-infiltrating CD3+CD56+ natural killer T cells in the pathogenesis of nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2009; **21**: 673-680 [PMID: 19318971 DOI: 10.1097/MEG.0b013e32831bc3d6]

21 **Guebre-Xabier M**, Yang S, Lin HZ, Schwenk R, Krzych U, Diehl AM. Altered hepatic lymphocyte subpopulations in obesity-related murine fatty livers: potential mechanism for sensitization to liver damage. *Hepatology* 2000; **31**: 633-640 [PMID: 10706553 DOI: 10.1002/hep.510310313]

22 **Li Z**, Soloski MJ, Diehl AM. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 880-885 [PMID: 16175608 DOI: 10.1002/hep.20826]

23 **Adler M**, Taylor S, Okebugwu K, Yee H, Fielding C, Fielding G, Poles M. Intrahepatic natural killer T cell populations are increased in human hepatic steatosis. *World J Gastroenterol* 2011; **17**: 1725-1731 [PMID: 21483633 DOI: 10.3748/wjg.v17.i13.1725]

24 **Syn WK**, Agboola KM, Swiderska M, Michelotti GA, Liaskou E, Pang H, Xie G, Philips G, Chan IS, Karaca GF, Pereira Tde A, Chen Y, Mi Z, Kuo PC, Choi SS, Guy CD, Abdelmalek MF, Diehl AM. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 2012; **61**: 1323-1329 [PMID: 22427237 DOI: 10.1136/gutjnl-2011-301857]

25 **Thomson AW**, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* 2010; **10**: 753-766 [PMID: 20972472 DOI: 10.1038/nri2858]

26 **Rocha VZ**, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, Libby P. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circ Res* 2008; **103**: 467-476 [PMID: 18658050 DOI: 10.1161/CIRCRESAHA.108.177105]

27 **Feuerer M**, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* 2009; **15**: 930-939 [PMID: 19633656 DOI: 10.1038/nm.2002]

28 **De Rosa V**, Procaccini C, Calì G, Pirozzi G, Fontana S, Zappacosta S, La Cava A, Matarese G. A key role of leptin in the control of regulatory T cell proliferation. *Immunity* 2007; **26**: 241-255 [PMID: 17307705 DOI: 10.1016/j.immuni.2007.01.011]

29 **Eller K**, Kirsch A, Wolf AM, Sopper S, Tagwerker A, Stanzl U, Wolf D, Patsch W, Rosenkranz AR, Eller P. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* 2011; **60**: 2954-2962 [PMID: 21911743 DOI: 10.2337/db11-0358]

30 **Zeng C**, Shi X, Zhang B, Liu H, Zhang L, Ding W, Zhao Y. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. *J Mol Med* (Berl) 2012; **90**: 175-186 [PMID: 21964948 DOI: 10.1007/s00109-011-0816-5]

31 **Zúñiga LA**, Shen WJ, Joyce-Shaikh B, Pyatnova EA, Richards AG, Thom C, Andrade SM, Cua DJ, Kraemer FB, Butcher EC. IL-17 regulates adipogenesis, glucose homeostasis, and obesity. *J Immunol* 2010; **185**: 6947-6959 [PMID: 21037091 DOI: 10.4049/jimmunol.1001269]

32 **Lynch L**, Nowak M, Varghese B, Clark J, Hogan AE, Toxavidis V, Balk SP, O'Shea D, O'Farrelly C, Exley MA. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* 2012; **37**: 574-587 [PMID: 22981538 DOI: 10.1016/j.immuni.2012.06.016]

33 **Bertola A**, Ciucci T, Rousseau D, Bourlier V, Duffaut C, Bonnafous S, Blin-Wakkach C, Anty R, Iannelli A, Gugenheim J, Tran A, Bouloumié A, Gual P, Wakkach A. Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 2012; **61**: 2238-2247 [PMID: 22596049 DOI: 10.2337/db11-1274]

34 **Winer DA**, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med* 2011; **17**: 610-617 [PMID: 21499269 DOI: 10.1038/nm.2353]

35 **Lumeng CN**, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 2008; **57**: 3239-3246 [PMID: 18829989 DOI: 10.2337/db08-0872]

36 **Tsochatzis EA**, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. *Mediators Inflamm* 2009; **2009**: 831670 [PMID: 19753129 DOI: 10.1155/2009/831670]

37 **Gonciarz M**, Bielański W, Partyka R, Brzozowski T, Konturek PC, Eszyk J, Celiński K, Reiter RJ, Konturek SJ. Plasma insulin, leptin, adiponectin, resistin, ghrelin, and melatonin in nonalcoholic steatohepatitis patients treated with melatonin. *J Pineal Res* 2013; **54**: 154-161 [PMID: 22804755 DOI: 10.1111/j.1600-079X.2012.01023.x]

38 **Kamada Y**, Takehara T, Hayashi N. Adipocytokines and liver disease. *J Gastroenterol* 2008; **43**: 811-822 [PMID: 19012034 DOI: 10.1007/s00535-008-2213-6]

39 **Lord GM**, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897-901 [PMID: 9732873 DOI: 10.1038/29795]

40 **Procaccini C**, Jirillo E, Matarese G. Leptin as an immunomodulator. *Mol Aspects Med* 2012; **33**: 35-45 [PMID: 22040697 DOI: 10.1016/j.mam.2011.10.012]

41 **Matsumoto H**, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, Hayashi N. Adiponectin deficiency exacerbates lipopolysaccharide/D-galactosamine-induced liver injury in mice. *World J Gastroenterol* 2006; **12**: 3352-3358 [PMID: 16733851]

42 **Yokota T**, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000; **96**: 1723-1732 [PMID: 10961870]

43 **Marchesini G**, Pagotto U, Bugianesi E, De Iasio R, Manini R, Vanni E, Pasquali R, Melchionda N, Rizzetto M. Low ghrelin concentrations in nonalcoholic fatty liver disease are related to insulin resistance. *J Clin Endocrinol Metab* 2003; **88**: 5674-5679 [PMID: 14671152 DOI: 10.1210/jc.2003-031094]

44 **Pagano C**, Soardo G, Pilon C, Milocco C, Basan L, Milan G, Donnini D, Faggian D, Mussap M, Plebani M, Avellini C, Federspil G, Sechi LA, Vettor R. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. *J Clin Endocrinol Metab* 2006; **91**: 1081-1086 [PMID: 16394091 DOI: 10.1210/jc.2005-1056]

45 **El-Assal O**, Hong F, Kim WH, Radaeva S, Gao B. IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis: IL-6 protects against ethanol-induced oxidative stress and mitochondrial permeability transition in the liver. *Cell Mol Immunol* 2004; **1**: 205-211 [PMID: 16219169]

46 **Hardy T**, Anstee QM, Day CP. Nonalcoholic fatty liver disease: new treatments. *Curr Opin Gastroenterol* 2015; **31**: 175-183 [PMID: 25774446 DOI: 10.1097/MOG.0000000000000175]

47 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

48 **Okada-Iwabu M**, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, Yamaguchi M, Tanabe H, Kimura-Someya T, Shirouzu M, Ogata H, Tokuyama K, Ueki K, Nagano T, Tanaka A, Yokoyama S, Kadowaki T. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature* 2013; **503**: 493-499 [PMID: 24172895 DOI: 10.1038/nature12656]

49 **von Boehmer H**, Daniel C. Therapeutic opportunities for manipulating T(Reg) cells in autoimmunity and cancer. *Nat Rev Drug Discov* 2013; **12**: 51-63 [PMID: 23274471 DOI: 10.1038/nrd3683]

50 **Ilan Y**, Maron R, Tukpah AM, Maioli TU, Murugaiyan G, Yang K, Wu HY, Weiner HL. Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. *Proc Natl Acad Sci U S A* 2010; **107**: 9765-9770 [PMID: 20445103 DOI: 10.1073/pnas.0908771107]

51 **da Cunha AP**, Weiner HL. Induction of immunological tolerance by oral anti-CD3. *Clin Dev Immunol* 2012; **2012**: 425021 [PMID: 22162715 DOI: 10.1155/2012/425021]

52 **Mizrahi M**, Shabat Y, Ben Ya'acov A, Lalazar G, Adar T, Wong V, Muller B, Rawlin G, Ilan Y. Alleviation of insulin resistance and liver damage by oral administration of Imm124-E is mediated by increased Tregs and associated with increased serum GLP-1 and adiponectin: results of a phase I/II clinical trial in NASH. *J Inflamm Res* 2012; **5**: 141-150 [PMID: 23293533 DOI: 10.2147/JIR.S35227]

53 **Shabat Y**, Lichtenstein Y, Zolotarov L, Ben Ya'acov A, Ilan Y. Hepatoprotective effect of DT56a is associated with changes in natural killer T cells and regulatory T cells. *J Dig Dis* 2013; **14**: 84-92 [PMID: 23134214 DOI: 10.1111/1751-2980.12003]

54 **Roncarolo MG**, Battaglia M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat Rev Immunol* 2007; **7**: 585-598 [PMID: 17653126 DOI: 10.1038/nri2138]

55 **Cipolletta D**, Kolodin D, Benoist C, Mathis D. Tissular T(regs): a unique population of adipose-tissue-resident Foxp3+CD4+ T cells that impacts organismal metabolism. *Semin Immunol* 2011; **23**: 431-437 [PMID: 21724410 DOI: 10.1016/j.smim.2011.06.002]

56 **Lau P**, Fitzsimmons RL, Pearen MA, Watt MJ, Muscat GE. Homozygous staggerer (sg/sg) mice display improved insulin sensitivity and enhanced glucose uptake in skeletal muscle. *Diabetologia* 2011; **54**: 1169-1180 [PMID: 21279323 DOI: 10.1007/s00125-011-2046-3]

57 **Hoffstedt J**, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Löfgren P, Rydén M, Thörne A, Wirén M, Palmér M, Thorell A, Toft E, Arner P. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* 2010; **53**: 2496-2503 [PMID: 20830466 DOI: 10.1007/s00125-010-1889-3]

58 **Sin TK**, Yung BY, Siu PM. Modulation of SIRT1-Foxo1 signaling axis by resveratrol: implications in skeletal muscle aging and insulin resistance. *Cell Physiol Biochem* 2015; **35**: 541-552 [PMID: 25612477 DOI: 10.1159/000369718]

59 **Chen S**, Zhao X, Ran L, Wan J, Wang X, Qin Y, Shu F, Gao Y, Yuan L, Zhang Q, Mi M. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: a randomized controlled trial. *Dig Liver Dis* 2015; **47**: 226-232 [PMID: 25577300 DOI: 10.1016/j.dld.2014.11.015]

60 **Friedman SL.** Significant Anti-Fibrotic Activity of Cenicriviroc, A Dual CCR2/CCR5 Antagonist, in a Rat Model of Thioac- etamide-Induced Liver Fibrosis and Cirrhosis. *Hepatology* 2013; **58**: 1381A-1382A

61 **Lin ZH**, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011; **53**: 726-736 [PMID: 21319189 DOI: 10.1002/hep.24105]

62 **Thompson M,** Chang W, Jenkins H, Flynt A, Gottwald M, Lefebvre E. Improvements in APRI and FIB-4 fibrosis scores correlate with decreases in sCD14 in HIV-1 infected adults receiving cenicriviroc over 48 weeks. *Hepatology* 2014; **60**: 424A

63 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

64 **Weston CJ**, Shepherd EL, Claridge LC, Rantakari P, Curbishley SM, Tomlinson JW, Hubscher SG, Reynolds GM, Aalto K, Anstee QM, Jalkanen S, Salmi M, Smith DJ, Day CP, Adams DH. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. *J Clin Invest* 2015; **125**: 501-520 [PMID: 25562318 DOI: 10.1172/JCI73722]

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|  |  |  |
| --- | --- | --- |
| **Treatment** | **Target** | **Effect**  |
| anti-CD3 moAb  | CD3 | Reduction of liver enzymes and glucose and insulin levels  |
| Imm124-E | Treg stimulation  | Improvement of glucose metabolism parameters, lipid profile and liver injury  |
| Adoptive transfer |  |  |
|  Tregs  |  | Reduction of TNF-α-related inflammation |
|  CD4+ T cells |  | Reverses weight gain and insulin resistance  |
|  NKT cells |  | Decreases body fat, triglyceride levels, leptin levels, hepatic steatosis and insulin sensitivity  |
| RORγt ligands | RORγt | Th17 inhibition |
| Cenicriviroc | CCR2/5 inhibitor | Improvement of lipid metabolism and liver fibrosis |
| VAP-1 | Lymphocytes recruitment | Anti-inflammatory en anti-fibrogenic effect |

**Table 1 Treatment perspectives: novel agents acting on the immune system**

TNF: Tumour necrosis factor; ROR: RAR-related orphan receptor; CCR2: C-C chemokine receptor 2; VAP-1: Vascular adhesion protein-1; NKT: Natural killer T; Th17: T helper cell 17.