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CEACAM1 loss links inflammation to Insulin Resistance in obesity and Non-alcoholic Steatohepatitis (NASH)

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Abstract

Mounting epidemiological evidence points to an association between metabolic syndrome and non-alcoholic steatohepatitis (NASH), an increasingly recognized new epidemic. NASH pathologies include hepatocellular ballooning, lobular inflammation, hepatocellular injury, apoptosis and hepatic fibrosis. We will review the relationship between insulin resistance and inflammation in visceral obesity and NASH in an attempt to shed more light on the pathogenesis of these major metabolic diseases. Moreover, we will identify loss of the Carcinoembryonic antigen-related cell adhesion molecule 1 as a unifying mechanism linking the immunological and metabolic abnormalities in NASH.

Keywords

CEACAM1; Insulin resistance; Adipokines; Metabolic syndrome; NASH

Introduction

Metabolic syndrome, also known as insulin resistance, is a leading cause of mortality and morbidity in developed countries. It consists of a cluster of metabolic abnormalities that include visceral abdominal obesity, dyslipidemia and hypertension [1]. With visceral obesity involving activation of pro-inflammatory signaling pathways that adversely affect insulin action, metabolic syndrome has been increasingly characterized by a chronic sub-acute low-grade inflammatory state [2–8]. Moreover, growing epidemiological evidence supports the view that metabolic syndrome is also associated with non-alcoholic fatty liver disease (NAFLD) and its progressive form, non-alcoholic steatohepatitis (NASH). NASH pathologies include macrosteatosis as well as inflammation, apoptosis and fibrosis in liver. We will review in this article the relationship between insulin resistance and inflammation in visceral obesity and NASH. We will identify loss of Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1)-dependent pathways as common underpinning molecular mechanisms linking insulin resistance to the inflammatory and metabolic anomalies that characterize these metabolic diseases.

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Inflammation and insulin resistance in visceral obesity

Positive nutrient supply coupled with a sedentary lifestyle promotes obesity and insulin resistance. The visceral adipose tissue plays a central role in obesity and its associated increase in inflammatory state and insulin resistance. While visceral obesity may precede insulin resistance, the reverse could also occur. For instance, hyperinsulinemia caused by impaired hepatic insulin clearance, induces lipid production in liver and its subsequent redistribution to white adipose tissue to be stored, causing visceral obesity [9,10].

Conversely, ample evidence demonstrates that visceral adiposity causes systemic insulin resistance [11]. Increase in visceral adipose mass is associated with lipolysis and the release of free fatty acids (FFAs) [12,13]. It is also associated with elevated output of adipokines [14], resistin [15], and other modulators, which together with reduced adiponectin release [16,17] adversely affect insulin action in extra-adipocytic peripheral tissues, causing systemic insulin resistance.

Role of adipose tissue-derived adipokines in insulin resistance

Recruitment of macrophages and other inflammatory cells to adipose tissue—

The seminal studies of Hotamisligil et al. [18,19] initially identified the hypertrophic visceral adipose depot as a dynamic tissue releasing pro-inflammatory molecules in obese humans and rodents. Originally, this model pointed to obesity-derived metabolic stressors activating inflammation in adipocytes and resulting in insulin resistance in an autocrine fashion [20]. It was not until 2003, when several groups found that non-adipocytic cells are the main producers of pro-inflammatory factors in adipose tissue [21], and that these cells were bone marrow-derived macrophages, the expression of which was markedly induced during obesity [22,23].

Mounting evidence demonstrates that immune cells, including monocytes and macrophages, infiltrate metabolic tissues, such as the white fat depot and liver, to induce cytokine production and release during obesity [22–25]. Aiding the process of macrophage recruitment is the concomitant rise in the secretion of chemokines such as monocyte chemoattractant protein-1 (MCP-1, also known as C-C ligand2-CCL2) [26,2]. Other chemokines, such as CCL5 and CCL8, are also secreted from fat-laden adipocytes to attract circulating leukocytes, an event that further promotes the pro-inflammatory state in adipose tissue and eventually, in the whole system [27]. It is important to note that the level of these chemokines correlates positively with adipose tissue mass [28].

Macrophages can undergo an M1 or an M2 activation state, depending respectively, on whether pro-inflammatory or anti-inflammatory signals are elicited by metabolic cues. In the absence of obesity and fat accumulation, resident macrophages are at an M2 immunoregulatory anti-inflammatory state producing and secreting low amounts of immune modulators. As adiposity increases with excess energy supply, the adipose tissue-associated macrophage population increases and undergoes a shift to an M1 pro-inflammatory state (CD11c⁺) [22,23,5,6,29], releasing pro-inflammatory factors, such as tumor necrosis factor-alpha (TNFα) [30,4,8]. Additionally, tissue-associated lymphocytes switch from small immunosuppressive T regulatory cells (Foxp3⁺ CD4⁺ Treg)-dominated T helper 2 (Th2) to large and inflammatory CD8⁺-dominated Th1 helper cells (high CD8⁺/CD4⁺ ratio). More dendritic cells, B cells, mast cells and neutrophils infiltrate the hypertrophic adipose tissue, replacing innate invariant natural killer T cells (iNKT) and IL4-secreting eosinophils, and contributing to adiposity-induced changes in the inflammatory milieu [31,4,32,29].

Mechanistically, macrophage M1 polarization involves mainly the activation of the c Jun NH(2)-terminal kinase (JNK) inflammatory signaling pathways, as demonstrated by the

protective effect of selective JNK deletion in macrophages against diet-induced insulin resistance and tissue inflammatory infiltration [33].

Inflammatory role of adipokines—The hypertrophic white adipose tissue acts as an active endocrine organ that produces different hormones, chemokines and cytokines (adipokines) [34,5], some of which are pro-inflammatory, serving as negative regulators of insulin action [35–38] in humans [39,40] and rodents [41–45]. Pro-inflammatory adipokines include tumor necrosis factor- α (TNF α), interleukin-6 (IL6), IL1 β and inducible nitric oxide synthase (iNOS) [46–48]. They cause insulin resistance locally and systemically through multiple mechanisms. For instance, TNF α blunts insulin signaling by decreasing tyrosine phosphorylation of insulin receptor substrate (IRS) proteins through increasing their serine phosphorylation by JNK and the I kappa B kinase (IKK β) pathways [20,49–51,8,52,53]. Propagation of inflammatory signals in extra-adipocytic tissue, such as liver and the vascular system, involves activation of NF-kappa B (NF- κ B) transcriptional activity and induction of its target genes such as TNF α and MCP-1 pro-inflammatory factors [54,3].

TNF α : Emphasis on the significant role of inflammation in the pathogenesis of insulin resistance was based on the finding that infusion of TNF α has adverse systemic metabolic effects that lead to insulin resistance in rats [55], and that TNF α is derived from adipose tissue during obesity [18].

Under normal physiological conditions, circulating as well as adipose tissue-associated levels of TNF α are low. However, as adiposity increases, TNF α expression is markedly induced in rodents [23] and humans [19]. Adipose tissue-derived TNF α causes insulin resistance, hyperinsulinemia, and other metabolic anomalies via several mechanisms. These include: 1) Stimulation of lipolysis from white adipose tissue [56]; 2) Blunting IRS phosphorylation by the insulin receptor kinase in response to insulin [57]; and 3) Modulating inflammatory pathways to induce transcription/expression of adipokines with negative effect on insulin action (i.e. leptin) while repressing those with a protective effect (i.e. adiponectin) [58].

Interleukin-6: Interleukin 6 (IL6) acts as either a pro- or an anti-inflammatory cytokine, depending on cells and tissue of expression [59]. Its role in obesity and insulin resistance remains a subject of debate, despite a reported rise of its content in white adipose tissue of obese humans [60,61]. Peripheral administration of IL6 adversely affects insulin signaling, in part by inducing the suppressor of cytokine signaling 3 expression in hepatocytes [62]. Selective transgenic overexpression of constitutively active IKK β in hepatocytes causes an increase in NF- κ B activity and insulin resistance together with elevated hepatic and plasma IL6 levels. Conversely, administering an IL6 antibody ameliorates the insulin resistance state in these mice [54].

The pro-inflammatory function of IL6 has been challenged by the observations that mice with null deletion of this cytokine develop obesity, systemic insulin resistance, hepatosteatosis and hepatic inflammation [63,64], and that this cytokine can acutely exert an anti-inflammatory action in skeletal muscle during exercise. As surmised by several studies and reviewed recently [65], IL6 can exert differential site- and cell-specific inflammatory effect.

Moreover, chronically elevated levels of IL6 (as in conditions of elevated adiposity) exert a pro-inflammatory effect on insulin action in adipose tissue and liver, while acute rise of IL6 (i.e. following muscle contraction during exercise) applies an anti-inflammatory effect and improves insulin sensitivity. In support of the beneficial acute versus chronic effect of this cytokine, central administration of IL6 decreases obesity by enhancing energy expenditure

[66], while its transgenic overexpression in muscle leads to insulin resistance and systemic hyperinsulinemia in addition to a marked inflammatory phenotype in liver [67].

Leptin: Leptin is a 16 kDa-peptide that is produced mainly by mature adipocytes. Its structure is similar to that of IL6 and other pro-inflammatory helical cytokines. Leptin induces inflammatory responses upon binding to the long isoform of its receptor and activating the Janus kinase 2 and signal transducer and activator of transcription 3 signaling pathway.

In addition to regulating food intake and energy expenditure [68,69], leptin regulates the immunological response to diet [70,69]. For instance, it promotes the phagocytic activity of macrophages/monocytes by activating phospholipase, and their production/secretion of pro-inflammatory cytokines, such as TNF α , IL6, and IL12 [71]. Leptin induces the production of CCL2 in human hepatic stellate cells [72]. It also induces the release of pro-inflammatory Th1 cytokines and suppresses that of anti-inflammatory Th2 cytokines [71]. Reciprocally, TNF α stimulates the protein content of leptin and the surface expression of its receptor [73].

Deficiency in leptin (as in *Ob/Ob* mice) and its receptor (as in *db/db* mice) results in obesity and insulin resistance, largely due to hyperphagia. On the other hand, obese humans and rodents exhibit chronically elevated levels of leptin (leptinemia) and leptin resistance, similarly to the development of hyperinsulinemia and insulin resistance in this metabolic condition (reviewed in [29]).

Adiponectin: In addition to increased expression of pro-inflammatory cytokines (i.e. TNF α), obesity is generally associated with reduced expression of anti-inflammatory factors (e.g., adiponectin, IL10) [74,17]. Adiponectin is secreted mainly from white adipose tissue to protect insulin action on fat and glucose metabolism, not only locally, but also outside the adipocytes, namely in liver, vascular tissues and others [17]. This is mediated in part by activating AMP-dependent protein kinase to enhance fatty acid oxidation and glucose uptake in skeletal muscle and decrease glucose production in liver [75]. Adiponectin levels are low in the adipose tissue of obese rodents and humans [76], due to the suppressive activity of inflammatory factors such as TNF α , IL6, reactive oxygen species (ROS), and hypoxia. In contrast, agonists of the peroxisome proliferator-activated receptor gamma (PPAR γ) induce its expression [77].

Neither the insulin sensitizing effect of adiponectin nor its anti-inflammatory effect in both humans and rodents is disputable. The mechanistic underpinning of adiponectin function includes: 1) Promoting the M2 anti-inflammatory polarization of macrophages and their phagocytic activity against apoptotic cells [78,79]; 2) Modulating T cells activation by inducing adiponectin receptors expression on the surface of T cells after antigen stimulation, followed by apoptosis-mediated suppression of antigen specific T cells expansion [80]; 3) Modulating the inflammatory function of natural killer (NK) cells by suppressing their Toll Like Receptor (TLR)-mediated interferon gamma (IFN γ) production without affecting their cytotoxicity [80]; 4) Suppressing transcription of TNF α , IL6 and other pro-inflammatory factors by inhibiting the IKK β /NF- κ B signaling inflammatory pathways [81–84]; 5) Increasing PPAR γ 2 expression in adipocytes [82]; and 6) Inducing IL10 expression [85,86].

Role of visceral adipose tissue-derived fatty acids in insulin resistance

In addition to secreting adipokines, visceral adipose tissue also releases FFAs that play a major role in the systemic insulin resistance that develops in response to prolonged positive nutrient supply.

Insulin resistance in white adipose tissue is characterized by increased lipolysis (i.e. fatty acid mobilization into the plasma). The majority of obese and insulin resistant individuals have elevated levels of plasma FFAs [87,88] that predispose them to glucose intolerance and progression to type 2 diabetes independently of other factors [89–91]. Being less sensitive to the anti-lipolytic effect of insulin than subcutaneous fat, visceral fat supplies higher levels of FFAs [92,93]. Displaced FFA are preferentially removed by PPAR α -mediated β oxidation in liver [94], and to a much lower extent in muscle, heart and others [93]. According to the Portal Hypothesis [95,96], mobilized FFAs reach the liver primarily via the portal vein and contribute to insulin resistance [97,13,98,99] by promoting gluconeogenesis [100,101], fatty acid oxidation and lipogenesis [102,103], inhibiting insulin-mediated suppression of glycogenolysis [104] and impairing hepatic insulin clearance to cause chronic hyperinsulinemia [105]. At the skeletal muscle level, increased FFAs uptake followed by oxidation competes with glucose oxidation and leads to insulin resistance (Glucose-Fatty Acid Cycle) [106].

Increased ectopic deposition of fat in liver, skeletal muscle and vasculature [107,108] elevates the intracellular content of FFAs and their metabolites to activate signaling pathways that impair insulin signaling via multiple mechanisms. These include activation of protein kinase C (PKC) epsilon and lambda [109], and engaging inflammatory signals [87] that activate the JNK [110] and the IKK β /NF- κ B pathways, leading to increased expression of inflammatory cytokines such as TNF α [110].

Inflammation and insulin resistance in the pathogenesis of NAFLD/NASH

NAFLD is a common chronic liver disease worldwide [111]. Clinically, the disease ranges from simple steatosis to fibrosing steatohepatitis and progressive non-alcoholic steatohepatitis (NASH). NAFLD affects more than one-third of adults in industrialized countries [112]. In the United States, 20–30% of NAFLD cases develop fibrosing steatohepatitis, of which ~10% progress to NASH. Furthermore, ~20% of patients with NASH develop cirrhosis [113]. NASH is a leading cause of cirrhosis, hepatocellular carcinoma [114,115,112,116] and end-stage liver disease [116]. Thus, the disease is expected to become the primary indication for liver transplantation in Western countries [117].

The features of NASH are: hepatic macrosteatosis, hepatocyte ballooning, inflammation, fibrosis, hepatocyte damage, and apoptosis [118,119,111,120]. The pathogenesis remains partly elucidated, with attendant diagnostic uncertainty [121]. However, the most common underlying “two-hit” hypothesis [122,123] suggests that fat accumulation in liver is the first hit, causing lipotoxicity that elicits adaptive intracellular signaling and apoptotic mechanisms mediated by lipid peroxidation, oxidative stress, inflammation, loss of hepatocytes by apoptosis, and fibrosis (second hit).

NASH and metabolic syndrome

NAFLD is strongly associated with obesity and metabolic syndrome [124,125]. In fact, NAFLD is regarded as the hepatic manifestation of the metabolic syndrome, as has been pointed out by several groups [124,126]. Mounting epidemiological evidence connects the growth of the incidence of NAFLD to increased prevalence of metabolic syndrome [111]. Moreover, it has been widely accepted that treating metabolic syndrome improves liver function in patients with NAFLD.

The metabolic syndrome is comprised of a constellation of finding that include obesity, dyslipidemia, hypertension, and insulin resistance, as indicated by fasting hyperinsulinemia, impaired glucose tolerance, and reduced glucose disposal in a glucose clamp. It is puzzling

that the biochemical, cellular, and genetic underpinnings of the linkage between hepatic insulin resistance to NASH have not been identified yet [127,128]. This possibly reflects the diversity of metabolic, inflammatory, fibrogenic and cell survival mechanisms implicated in the pathogenesis of NASH. A key hurdle is that insulin affects these processes in different ways: for example, in NASH, hepatocyte survival is reduced, indicating resistance to the pro-survival actions of insulin. In contrast, lipogenesis is increased, consistent with the lipogenic actions of insulin [11,129–131].

Role of Insulin Resistance in the pathogenesis of NASH

Metabolic abnormalities, such as obesity and metabolic syndrome, are characterized by insulin resistance [132] and are commonly accepted as risk factors of NASH [133]. Thus, it is reasonable to link insulin resistance to NAFLD/NASH [124,134].

Hepatic insulin resistance is synonymous with a defective ability of insulin to suppress glycogenolysis and gluconeogenesis, thus causing elevated hepatic glucose production. Insulin resistance and hyperinsulinemia favor fat accumulation in liver by promoting *de novo* lipogenesis [130,131], at least partly by inducing the expression of lipogenic genes by the nuclear sterol regulatory element-binding protein 1c (nSREBP-1c), a master regulator of the transcription of lipogenic enzymes [135]. Insulin resistance can also elevate fat accumulation in liver by increasing fatty acids mobilization from adipose tissue to be redistributed to the liver where it decreases their mitochondrial β -oxidation [136].

Debate over the role of insulin resistance in the pathogenesis of NAFLD/NASH persists [128,137]. This unresolved question could, at least in part, be attributed to the paucity of animal models that manifest insulin resistance while replicating adequately all features of the human disease [138]. The historic lack of such animal models has prevented a sustained research effort in this area. There is a dearth of animal models that recapitulate the features of NASH [137,139]. Among the most commonly employed models are *Ob/Ob* obese mice [140], inositol-phosphatase *Pten* null mice [141], adipose-tissue nSREBP-1c transgenic mice [142], KK- A^y mice [143], and mice with null mutation of hepatic PPAR α [144]. The leptin-deficient *Ob/Ob* mice are extremely resistant to insulin, in contrast to the more moderate insulin resistance state in the human disease, and they exhibit altered leptin signaling, which contrasts to the permissive effect of leptin on inflammation, fibrosis and lipogenesis [140]. There are also limitations to the use of the liver-specific *Pten* knockout mice, owing to their insulin sensitivity and leanness, as well as a degree of steatosis that is typically not associated with human NASH [141]. The adipose tissue nSREBP-1c transgenic mouse displays a NASH-like liver histology [142], but exhibits lipodystrophy with hypoleptinemia together with severe insulin resistance that do not fully replicate the clinical manifestation of NASH.

Some diets can similarly induce NASH phenotype, or at least part of it. The most widely used to induce fibrosis is the methionine and choline deficient (MCD) diet. However, humans with NASH do not exhibit methionine or choline deficiency nor does this diet cause insulin resistance [145]. Diets rich in high-fat [146], cholesterol and cholate [147], and fructose [137,148,139,149] have also been commonly used. By not truly mimicking either the biochemical or the full clinical manifestation of NASH, these genetic and dietary models have collectively failed to adequately probe the role of insulin resistance in this disease process.

Role of Inflammation in the pathogenesis of NAFLD/NASH

Progression of NASH involves hepatic lipid accumulation that contributes to a pro-inflammatory state in liver. Lipotoxic signals activate kupffer cells (the resident

macrophages in liver) to release pro-inflammatory molecules and chemokines that recruit other inflammatory cells, and contribute to the production of fibrogenic mediators and activation of stellate cells, causing hepatic fibrosis. NASH progression also involves hepatocellular injury that results from hepatocyte apoptosis, oxidative stress and endoplasmic reticulum (ER) stress. Additionally, a growing body of work implicates extra-hepatic cellular events in the progression of NASH. Among these is the release of FFAs and pro-inflammatory adipokines from white adipose tissue. Next, we will review the major extra-hepatic and local inflammatory mechanisms that are involved in NAFLD progression to NASH.

Hepatic mechanisms involved in the inflammatory phenotype and hepatocellular injury in NASH—

Visceral obesity and insulin resistance contribute to the development of several metabolic (type 2 diabetes and NAFLD) and cardiovascular diseases [150,151]. Under conditions of systemic insulin resistance, FFAs are released from white adipose tissue to be distributed to other organs, including liver and the vascular system. Moreover, the liver is equipped with lipogenic enzymes that are induced by hyperinsulinemia [135]. Hence, increased fat accumulation in liver can be attributed to lipolysis, but also to elevated hepatic *do novo* lipogenesis, in particular under conditions of chronic hyperinsulinemia.

Ectopic fat accumulation in liver alters the inflammatory milieu, at least partly, by causing an increase in the local expression of pro-inflammatory genes that could exacerbate NAFLD and insulin resistance [54]. The liver harbors several immune cells such as lymphocytes, NK cells, and dendritic cells that take part in NASH inflammatory process [2]. Profiling of genetic changes during progression from obesity to NASH identified a liver-specific genetic signature in NASH patients [152]. Several genes were found to be upregulated in the liver of patients with NASH by comparison to patients with NAFLD or morbid obesity. These include genes encoding chemokines and their receptors, which are involved in leukocyte recruitment, CDs (most notably, CD62E/E-Selectin; CD69/EA1, CD54/ICAM1, and CD11b/ITGAM), and cytokines that are involved in steering T cell activation towards a Th1 phenotype. In contrast, changes in the serum and visceral adipose tissue immune map, such as increased levels of MCP1, TNF α and IL6 levels, were independent of NASH, and were also detected in patients with simple NAFLD. Similar results on the dominance of an IFN γ -producing Th1 cytokine profile were also reported in the liver of obese children with NASH pathologies [153].

Under conditions of obesity and prolonged high fat intake, the liver boosts its fatty acid β -oxidation as a compensatory mechanism to limit fat accumulation [154]. Oxidative stress can result from unchecked fatty acid oxidation and lipid ω -peroxidation [155]. When this occurs together with reduction of mitochondrial glutathione (GSH) oxidation, it may lead to activation of the NF- κ B inflammatory pathway and indirectly cause insulin resistance and mitochondrial dysfunction [156]. This sequence of events in the liver can cause susceptibility to injury, cell death [157], hepatitis [158,159], and progressive liver disease [160]. In addition to oxidative stress, IKK β /NF- κ B inflammatory pathways are involved in the activation of unfolded protein response (UPR) by ER stress that is linked to metabolic abnormalities in obesity, type 2 diabetes [161,51], and hepatic steatosis [162,163].

Moreover, signaling through JNK1, a mediator of TNF α -induced apoptosis, appears to regulate hepatic steatosis and insulin resistance, as bolstered by the observation that deleting JNK1 protects against steatohepatitis [164] and diet-induced rise in the inflammatory state and insulin resistance in liver [165]. Moreover, mice with hepatocyte-specific but not hematopoietic-loss of JNK1/2 exhibit hepatitis [166]. Because mice with specific loss of

JNK1/2 in hematopoietic cells manifest a marked decrease in TNF α level, this shows that hematopoietic cells are the principal regulators of TNF α expression and JNK function [166].

Role of resident macrophages (Kupffer Cells) in NAFLD/NASH: In addition to hepatocytes, the liver contains a large number of resident macrophages, known as kupffer cells, that constitute about 20% of hepatic non-parenchymal cells [167]. These cells are phagocytic and when activated, produce cytokines (TNF α , IL-1, IL-6) as well as chemokines (CXCL1-3, CXCL-8, CCL2-4) that initiate inflammation and induce cell death [168].

Kupffer cells are implicated in the liver's response to infection, toxins and other stressors, including fat accumulation. However, their role in hepatic steatosis and NAFLD is not fully delineated [169]. In general, adiposity affects the immune cellular response of the liver in a way similar to white adipose tissue (see above) [54], with a relatively more limited increase in macrophage recruitment [170,171]. Moreover, the population of macrophage in NASH exhibits an M1 bias in C57BL/6 mice, in support of this strain's favorable disposition to the disease [172].

Patients with NAFLD display an increase in intestinal permeability that could elevate circulating lipopolysaccharide (LPS) [173], which activate all cell types in liver, including kupffer cells, to produce reactive oxygen species (ROS) and pro-inflammatory cytokines, such as TNF α . This causes liver injury in NASH [174], as TNF α induces insulin resistance and ROS promotes lipid peroxidation and oxidative stress [175].

Furthermore, activated kupffer cells can produce transforming growth factor- β 1 (TGF- β 1) [169], which in turn, contributes to the activation of hepatic stellate cells, the main cells for collagen production in liver, to mediate extracellular matrix deposition and promote fibrosis. This points to a role for macrophages and inflammatory pathways in hepatic fibrosis [176]. Indeed, patients with NAFLD develop IgG antibodies against lipid peroxidation-derived antigens in association with advanced fibrosis [177]. Thus, activated kupffer cells contribute to numerous processes in NASH progression: steatosis, apoptosis, inflammation and fibrosis.

Role of lymphocytes in NAFLD/NASH: The liver is also home to NK cells as well as natural killer T (NKT) cells, a unique subset of T regulatory lymphocytes in the innate immune system. NK cells modulate hepatic T-cells response in addition to directly promoting death of hepatocytes and activated stellate cells [178]. NKT cells are implicated in liver injury; their primary function is to mediate a balance between local production of Th1 pro-inflammatory and Th2 anti-inflammatory cytokines [179]. IFN γ , IL12, TNF α and TNF β are among the Th1 pro-inflammatory/anti-fibrogenic cytokines released by NKT cells, and IL4, IL10 and IL13 are among those of the Th2 anti-inflammatory/pro-fibrogenic response [180]. NASH is associated with a relative imbalance favoring a Th1 pro-inflammatory response [181,182].

NKT cells are especially abundant in liver. Patients with NAFLD manifest a decrease in peripheral NKT cell number [183], owing in part, to cell death by kupffer cells-derived IL12 pro-inflammatory cytokines [184]. In *Ob/Ob* mice, the pool of NKT cells decreases in correlation with elevated hepatic production of Th1 cytokines and severe steatosis [185]. Adoptive transfer of IL4 producing regulatory NKT cells ameliorates steatosis, normalizes glucose homeostasis and decreases levels of Th1 pro-inflammatory cytokines in these mice [185]. On the other hand, some studies showed that NKT population increases rather than decreases during NAFLD progression to late-stage disease, proposing a potential role for NKT in more advanced pathologies, such as fibrosis [186,187].

Role of adipose tissue-derived adipokines in NASH—Clinical studies have shown that white visceral fat is a key mediator of liver inflammation and fibrosis independently of the metabolic abnormalities of NASH (insulin resistance and hepatic steatosis) [188]. Even though the exact role of adipokines in hepatic insulin resistance and NAFLD remains to be fully elucidated, altered adipokines patterns have been observed in patients developing insulin resistance [2] as well as in obese patients with NAFLD [189,190].

The visceral adipose tissue is hormonally active and produces several adipokines that contribute to insulin resistance and altered glucose and fat metabolism in the liver. Adipokines undergo similar changes in obese patients with insulin resistance and NAFLD, independently of NASH. Nonetheless, they contribute not only to the insulin resistance state associated with the disease, but also to macrophage recruitment to the liver, activation of its resident macrophages and the fibrogenic activity of stellate cells; all constituting critical components of NASH pathogenesis.

TNF α : In addition to its role in the development of insulin resistance, TNF α plays a key role in the initiation of NAFLD, as well as its progression to NASH in rodents and humans and in the development of fibrosis [190]. During progression to NASH, activated kupffer cells become a major supplier of TNF α . Treatment with an antibody against TNF α reduces the activity of JNK and NF- κ B pathways and prevents the progression of NAFLD into NASH in *Ob/Ob* mice [191]. Patients with steatohepatitis exhibit elevation in plasma [192] as well as tissue-associated levels of TNF α and its receptors [193]. The increase in TNF α has been shown to positively correlate with advanced stage of the disease, including the degree of hepatic fibrosis [194,195].

IL-6: The role of IL6 in the pathogenesis of NAFLD remains unclear. Several clinical studies show that hepatocyte IL6 expression correlates positively with plasma IL6 levels and the degree of inflammation and fibrosis in patients with NASH [196,197,188]. More specifically, IL6 levels, which are directly associated with elevated visceral fat, independently predict the degree of liver inflammation in patients with NASH [188]. In addition to macrophages, IL6 could be released from B cells, as part of the adaptive immune response [198], to induce differentiation of hepatic stellate cells to myofibroblasts and mediate hepatic fibrosis in response to CCl4 in mice, independently of T-cell or antibody stimulation [199]. However, the pro-inflammatory effect of IL6 has been challenged by the observations that it can also play an anti-inflammatory role [65]. For instance, some studies show that IL6 exerts a protective mechanism against liver fibrosis by promoting hepatocyte regeneration and proliferation [200], and preventing oxidative stress and mitochondrial dysfunction [201]. Consistently, blocking IL6 signaling exacerbates hepatocyte apoptosis, liver injury and fibrosis in MCD fed-*db/db* diabetic mice, a model that presents severe NASH pathologies [202]. Thus, further studies are needed to delineate the role of this cytokine in the pathogenesis of NASH.

Leptin: Leptin is released from white adipose tissue. Its levels are increased in the plasma of patients with NAFLD [203], suggesting that NAFLD is associated with leptin resistance in addition to insulin resistance [204].

The role of leptin in regulating energy expenditure and immune system function has been well documented (see above). Moreover, leptin has been shown to activate hepatic stellate cells [140], consistent with increased leptin receptor expression in these cells [205], and absence of hepatic fibrosis in leptin-deficient *Ob/Ob* mice [206].

It is important to note that serum leptin levels are not uniformly associated with advanced fibrosis [207,204], nor is the level of hepatic leptin receptor mRNA expression in humans

[208]. In contrast to NASH, patients with cirrhosis may exhibit elevated serum leptin levels [209]. Hence, more studies are needed to dissect out the role of leptin in fibrosis and NASH progression.

Adiponectin: In contrast to leptin, adiponectin levels are lower in patients developing steatosis [192], and in patients with NASH or with more severe liver injury [210,211]. Furthermore, adiponectin plasma levels are inversely associated with plasma levels of IL-6 and TNF α [212–214]. In contrast to NASH, patients with cirrhotic liver disease manifest an increase in adiponectin levels [215].

Regardless, adiponectin has been shown to suppress alcoholic as well as non-alcoholic fatty liver disease and liver fibrosis in mice [216], and to suppress ER stress in transgenic mice overexpressing nSREBP-1c in adipose tissue, a mouse model of NASH [217]. Thus, it is likely that adiponectin has beneficial effect on this metabolic disease.

Loss of CEACAM1 causes insulin resistance, visceral obesity, and fibrosing steatohepatitis in mice

CEACAM1 structure and topology

CEACAM1 (previously called biliary glycoprotein [BGP], C-CAM1, CD66a or pp120/HA4) is the most highly conserved and most broadly distributed member of the CEA molecules. It is expressed in most epithelial cells, endothelial, lymphoid, and myeloid cells [218].

CEACAM1 is a type I membrane glycoprotein with a single transmembrane domain. In 1993, Najjar et al. [219] initially showed that Ceacam1 gene consists of 9 exons, the 7th of which undergoes alternative splicing to generate either a long (71–73 a.a depending on species) or a short (10–12a.a) intracellular tail. Subsequently, the mouse and human genes were found to undergo similar alternative splicing events. It is important to note that the cytoplasmic tail is highly conserved among all species.

The full-length protein consists of a membrane-distal Ig variable domain (IgV)-like amino (N)-terminal domain region that is highly conserved among all CEA family members, followed by 1 to 3 membrane-proximal C2-type Ig constant domain-related sequences. The N-domain mediates homophilic intercellular adhesion. For simplicity, we will in this review refer to the functions of the long isoform that contains two tyrosine phosphorylation sites in its cytoplasmic tail and harbors all the regulatory mechanisms of the CEACAM1 functions pertaining to insulin resistance and NASH pathogenesis; namely, its role to suppress inflammation and steatosis and to promote insulin action in liver.

CEACAM1 is expressed predominantly as a L-*cis*-dimer on the cell membrane of immune cells, where it can also engage in trans-homophilic (CEACAM1–CEACAM1) and trans-heterophilic (CEACAM1–CEACAM5) intercellular binding as monomers [220].

CEACAM1 and regulation of the inflammatory response

CEACAM1 in lymphoid cells—As reviewed in [220,221], CEACAM1 expression is low in resting T and NK cells from mice and humans. Upon stimulation, it undergoes rapid mobilization from an intracellular compartment (via lipid rafts) to become strongly upregulated on the cell surface of all classes of CD4⁺ and CD8⁺ T cells [222–225]. On the surface membrane, CEACAM1 acts as a co-inhibitory receptor of the T cell receptor (TCR)/CD3 complex through homophilic interactions, especially if the antigen-presenting cell expresses CEACAM1, such as B cell, dendritic cell and macrophage. This results in

inhibiting cytokine production (mostly IFN γ -dominant Th1) and cytotoxicity in NK cells via a class I MHC-independent inhibitory mechanism [226–228].

By binding to *Src* homology domain phosphatase (SHP-1), human CEACAM1 mediates the association between the TCR/CD3 complex with ZAP70 and calmodulin to enter the immune synapse [223]. Mechanistically, TCR activation stimulates CEACAM1 phosphorylation by the *src*-kinases, *lck*, and *fyn* on its cytoplasmic tail. Tyrosine phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) (I/VxYxxL/V) stabilizes CEACAM1 monomers on the cell surface and mediates its association with SHP-1, which in turn, dephosphorylates *src*- and *syk*-kinases [229,230], in particular, the *syk*-related kinase, ZAP70, a major regulator of the integration of TCR signaling [230].

In primary spleen B cells, CEACAM1 is constitutively expressed [231]. In these cells, CEACAM1 may act either co-stimulatory [232] or co-inhibitory [233]. Recent evidence shows that similar to its negative role on TCR signaling in T cells, CEACAM1 acts as a negative co-receptor to B cell receptor in human cells [234].

CEACAM1 in blast/myeloid cells—Whereas CEACAM1 is expressed on dendritic cells [235], its function in these cells remains to be determined. In contrast, its function in neutrophils has been more thoroughly investigated [236]. CEACAM1 is highly expressed in neutrophils [237], the most abundant leukocytes. Neutrophils mediate inflammation by producing chemokines and cytokines, such as IL1 β . CEACAM1 reduces IL1 β production in LPS-activated neutrophils by recruiting SHP-1 phosphatase to the complex formed between phosphorylated Syk and TLR4, downregulating TLR4 activity and inhibiting inflammasome activation [236]. This key inhibitory co-receptor role for CEACAM1 in neutrophils, coupled with its relatively higher expression in these cells than in monocytes and macrophages [238], is consistent with the higher demand to negatively regulate the inflammasome in neutrophils, which are dominant in murine bone marrow. Nonetheless, it is reasonable to predict that CEACAM1 applies a similar anti-inflammatory effect in macrophages and monocytes.

CEACAM1 and regulation of insulin action and fat metabolism

Upon its release from pancreatic β -cells, insulin reaches the liver via the portal circulation to undergo rapid clearance. ~ 50% of insulin is cleared during its first pass through the liver. This process involves intracellular uptake of insulin via its receptor into clathrin-coated pits and vesicles [239]. In the acidic environment of endosomes, insulin dissociates from its receptor to undergo degradation by insulin degrading enzymes, and the receptor undergoes recycling to the membrane. Of note, insulin clearance mainly occurs in the liver, and to a lower extent, in the kidney [reviewed in [240]].

We have shown that CEACAM1 undergoes phosphorylation by the insulin receptor tyrosine kinase on tyrosine 408 (numbering applies to the rat protein), and that this phosphorylation requires an intact serine 503 residue in its cytoplasmic tail [241]. Upon its phosphorylation, CEACAM1 takes part of insulin-receptor complex [242] to increase the rate of receptor-mediated insulin endocytosis and degradation in the hepatocyte [243]. This concomitant increase in CEACAM1 entry into the cell promotes its association with fatty acid synthase (FAS), a critical enzyme in *de novo* lipogenesis in liver [244]. This causes its detachment from the insulin-receptor complex to promote its destabilization and subsequently, insulin dissociation from its receptor followed by its degradation. CEACAM1 binding to FAS mediates an acute inhibitory effect on FAS enzymatic activity by insulin [244]. This acute negative effect of insulin on FAS activity is mediated by the pulsatility of insulin outflow into the portal vein from pancreatic β -cells [245]. Moreover, it is consistent with the physiologically low levels of FAS activity in liver. Thus, we propose that CEACAM1

phosphorylation by the acute rise of insulin pulses bestows on the liver a protective mechanism against the normally higher levels of insulin than in the systemic circulation [246]. Under conditions of obesity and hyperinsulinemia, when insulin secretion is induced and its pulsatility is reduced, phosphorylation of CEACAM1 is lost and subsequently, the negative acute effect of insulin gives way to its positive chronic effect on the transcription of lipogenic enzymes to induce *do novo* lipogenesis in liver [244]. This CEACAM1-dependent mechanism mediating an acute co-inhibitory effect on insulin signaling on lipogenesis promotes a unifying acute downregulatory effect of insulin on gluconeogenesis and lipogenesis.

Loss of CEACAM1 causes insulin resistance, hepatic steatosis and visceral obesity in mice

The regulatory effect of CEACAM1 on insulin internalization and degradation is bolstered by the observation that L-SACC1 mice with liver-specific dominant-negative transgenic inactivation of CEACAM1 harboring a non-phosphorylated serine 509 (S) to alanine (A) mutation, and mice with global null mutation of the Ceacam1 gene (*Cc1*^{-/-}) develop impairment of insulin clearance, which causes hyperinsulinemia [9,10,247]. Chronic hyperinsulinemia causes systemic insulin resistance, as shown by hyperinsulinemic-euglycemic clamp analyses [248,10,247]. It also induces transcription of lipogenic enzymes in liver (including FAS), resulting in increased hepatic lipid production and output, followed by substrate redistribution to white adipose tissue and eventually, visceral obesity [9,10,244].

Loss of CEACAM1 causes fibrosing steatohepatitis that advances to NASH in response to a prolonged high fat intake

Histological examination of liver section revealed that both of Ceacam1 mutants develop microsteatosis on a regular chow diet [249,250]. They also develop a pro-inflammatory state, marked by elevated tissue-associated macrophages, TNF α and IFN γ levels in liver, adipose tissue and aortae [251]. The population of hepatic CD4⁺ T cell was basally higher than wild type in *Cc1*^{-/-} [250], but not in L-SACC1 mice with functional inactivation of Ceacam1 in hepatocytes [249]. The similar increase in CD4⁺ T pool in mice with conditional Ceacam1 deletion in T cells [252], suggest that this rise is due, at least in part, to Ceacam1 loss in T cells, rather than hepatocytes. Elevation in hepatic mRNA content of IFN γ without changes in IL4/IL13 levels demonstrates a IFN γ -dominant CD4⁺ Th1 phenotype in *Cc1*^{-/-} null mice [253], as detected in obese children [153]. The rise in leptin and TNF α levels could contribute to its inflammatory phenotype [70]. Moreover, it is important to note that the null mouse exhibits spontaneous systemic neutrophilia with high hepatic Ly-6G⁺ CD11b⁺ population that results from neutrophil progenitor cell hyperproliferation [238]. Neutrophilia could also contribute strongly to the development of the pro-inflammatory state in Ceacam1 null mice.

Remarkably, Ceacam1 mutants also develop spontaneous pericellular fibrosis [249,250], consistent with insulin resistance being an independent predictor for fibrosis in NASH [254]. This finding reveals the uniqueness of this animal model as others fail to develop fibrosis on a regular diet [128]. Basal fibrosis in *Cc1*^{-/-} null mice could be attributed to elevated hepatic content of the pro-fibrogenic factors, IL6 and TGF β [255,198] despite the rise in TNF α , which together with IFN γ , could reduce collagen synthesis [198] and limit the pro-fibrogenic effect of IL6 and TGF β .

Collectively, this demonstrates that on a regular chow diet, Ceacam1 mutant mice spontaneously develop a state mimicking fibrosing steatohepatitis in humans, in addition to insulin resistance and visceral obesity.

Sustained high fat (HF) feeding, which triggers inflammation [256–258], caused several pathologic and metabolic alterations in *Ceacam1* mutants, similar to those detected in patients with progressive NASH [249,250]. Lipid accumulated in hepatocytes despite increased fatty acid β -oxidation [249]. Histologically, high-fat feeding caused more diffuse macrosteatosis in the liver [249,250]. It also reduced Niemann-Pick C protein (NPC-1) level, and consequently lowered GSH, presumably in mitochondria [259], which could theoretically enhance sensitivity to the cytotoxic effect of TNF α . Consistently, HF diet elevated TNF α dependent activation of IKK β /NF- κ B oxidative stress and inflammatory pathways [193].

High fat feeding caused progressive fibrosis in *Ceacam1* mutant mice with a NASH-characteristic chicken-wire fibrogenic deposition pattern [249,250]. This occurred in parallel to a further induction of TNF α and leptin levels without altering the tissue content of the anti-fibrogenic or pro-fibrogenic modulators, IFN γ or TGF β , respectively. Thus, it is plausible that the exaggerated fibrosis that developed in *Cc1*^{-/-} mice in response to HF feeding stems from an increase in TNF α -mediated apoptosis, which in turn, leads to fibrosis and inflammation [260,255,261]. By exacerbating the pro-fibrogenic effect of TNF α [194,262], elevated leptin could contribute to progressive fibrosis in response to HF feeding. It is important to note that in wild type mice, HF feeding elevated hepatic TGF β without causing NASH-like fibrogenic changes, possibly owing to failure of HF diet to alter IL6 expression in the liver of wild type mice.

Conclusions

In summary, liver-specific inactivation and global null mutation of *Ceacam1* cause systemic insulin resistance in addition to all clinical and biochemical features of fibrosing steatohepatitis (macrosteatosis, inflammation, apoptosis, necrosis and chicken-wire fibrosis) that progress to NASH in response to inflammatory cues elicited by a sustained high fat intake. Moreover, global *Ceacam1* deletion generates a pro-inflammatory state, typically detected in NASH patients, including a Th1 inflammatory phenotype and neutrophilia. Thus, *Ceacam1* mutant mice are indisputably reliable replicates of the human disease and provide a valuable tool to understand the molecular underpinning of NASH. The importance of our findings is highlighted by the recent report demonstrating a marked decrease of CEACAM1 levels in the liver of obese subjects with fatty liver disease, independently of diabetes [263].

The phenotype of *Cc1*^{-/-} mice is consistent with the pleotropic effects of CEACAM1 on all facets of the disease: promoting insulin clearance to maintain insulin sensitivity and prevent visceral obesity; mediating a co-inhibitory physiologic acute downregulatory effect of insulin on *de novo* synthesis of fatty acids in liver; and exerting a co-inhibitory effect on immune cells from the lymphoid as well as the myeloid lineage. Hence, loss of CEACAM1 constitutes a missing link between insulin resistance and changes in inflammatory and metabolic processes involved in the pathogenesis of visceral obesity and NASH. This uniquely identifies loss of CEACAM1 as a unifying mechanism linking the immunological and metabolic abnormalities in NASH, and promotes CEACAM1 as an important therapeutic target against this devastating disease.

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