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Beyond Insulin Resistance: Innate Immunity in Nonalcoholic Steatohepatitis

Jacquelyn J. Maher[1,4], **Pablo Leon**[2,3], and **James C. Ryan**[1,3]

[1]Department of Medicine, University of California, San Francisco, CA

[2]Department of Surgery, University of California, San Francisco, CA

[3]Veterans Affairs Medical Center, San Francisco, CA

[4]University of California San Francisco (UCSF) Liver Center, San Francisco, CA

Abstract

Obesity is an inflammatory disorder characterized by heightened activity of the innate immune system. Innate immune activation is central to the development of obesity-related insulin resistance; it also plays an important role in obesity-related tissue damage, such as that seen in atherosclerosis. Recent research has implicated the innate immune system in the pathophysiology of obesity-related liver disease. This review summarizes how innate immune processes, occurring both within and outside the liver, cause not only insulin resistance but also end-organ damage in the form of nonalcoholic fatty liver disease. (HEPATOLOGY 2008;48:670-678.)

Obesity is the direct result of an imbalance between nutritional intake and energy expenditure, which leads to the storage of excess fuel as fat. Although adipose tissue represents the body's principal lipid storage reservoir, fat can accumulate ectopically in other organs such as muscle and liver. Regardless of its location (even in adipose tissue), excess fat can provoke abnormalities in tissue structure and function that result in end-organ damage. A growing body of evidence supports the concept that end-organ damage in obesity is an inflammatory condition.[1] Consequences of this systemic inflammation include type 2 diabetes mellitus, atherosclerosis, and nonalcoholic fatty liver disease (NAFLD).[1] With respect to the liver, immune pathways can adversely affect hepatic lipid metabolism and lead to serious outcomes such as hepatic injury, inflammation, and fibrosis. These processes are likely at play in the 72 million obese adults in the United States (www.cdc.gov/nchs/pressroom/07newsreleases/obesity.htm), 75% of whom have fatty livers.[2]

The leukocytes, receptors, and soluble mediators involved in obesity-related inflammatory sequences are all part of the innate immune system. The evolutionary purpose of innate immunity is to defend against pathogens or foreign substances. In the setting of obesity, however, dietary fats or fatty acids may be perceived as foreign substances that modulate inflammation and its metabolic effects. Although a number of immune responses to fat can occur locally in target tissues, recent studies suggest a novel paradigm in which inflammation in adipose tissue is a master regulator of metabolic and immune dysfunction in other organs. In this context, an important question for the hepatologist is whether immune activation in adipose tissue is a prerequisite for the development of nonalcoholic

Address reprint requests to: Jacquelyn J. Maher, M.D., Rice Liver Center Laboratory, San Francisco General Hospital, 1001 Potrero Avenue, Building 40, Room 4102, San Francisco, CA 94110. jmaher@medsfgh.ucsf.edu; fax: 415-641-0517.

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steatohepatitis (NASH). Although data are currently incomplete, there is compelling evidence that adipose tissue inflammation exacerbates hepatic steatosis and can heighten innate inflammatory responses within the liver.[3,4] In this review, we will summarize evidence that innate immune pathways are activated in obesity and describe the involvement or proposed involvement of these pathways in the pathogenesis of NASH.

Fat-Induced Activation of Proinflammatory Pathways Causes Insulin Resistance

Fat can cause insulin resistance by prompting the activation of select serine kinases within a variety of insulin-sensitive cells. Singly or in combination, these enzymes phosphorylate regulatory serine residues on the insulin receptor substrates IRS-1 and IRS-2, leading to the down-modulation of normal insulin-stimulated tyrosine phosphorylation and interfering with physiologic insulin responses. A causal connection between fat-related activation of serine kinases and insulin resistance has been demonstrated in adipose tissue[5] and muscle[6,7] as well as liver.[8-11] It occurs quite rapidly *in vivo* in response to both intravenous fat infusion[6,8] and high-fat feeding.[9,10] Saturated as well as unsaturated fatty acids are capable of activating the serine kinases that lead to insulin resistance.[5,6,8,9,11,12] In the liver, long-chain saturated fatty acids appear to be the most potent species.[11,12]

The three serine kinases implicated most strongly in the pathogenesis of fat-induced insulin resistance are Jun N-terminal kinase (JNK), inhibitor of nuclear factor κB (NF-κB) kinase (IKK), and novel isoforms of protein kinase C (PKC).[13-15] Among these, JNK and IKK are particularly noteworthy proinflammatory signaling molecules. In the setting of obesity, activation of these kinases likely arises through several mechanisms as outlined in Fig. 1. In one scenario, PKC is activated by diacylglycerol formed during the intracellular metabolism of lipids,[14] with JNK and IKK being activated downstream of PKC as part of a signaling cascade.[5] In a second pathway, intracellular fat activates these kinases independently of PKC as part of an endoplasmic reticulum (ER) stress response.[16,17] A third pathway points to reactive oxygen species, which can be generated during fatty acid oxidation, as inducers of JNK and IKK[18] Fourth, extracellular fatty acids, by virtue of their resemblance to the lipid moieties of bacterial lipopolysaccharides, can activate IKK by engaging Toll-like receptors (TLRs).[19] The fact that all four of these pathways converge at the level of JNK and IKK points to the close interconnection between insulin resistance and inflammation in the setting of obesity. The complexity of the relationship is enhanced even further when one considers that inflammatory cytokines, induced by JNK and IKK, can contribute to a feed-forward amplification of insulin resistance and inflammatory signaling (see below). The pivotal role of inflammatory pathways in the pathogenesis of insulin resistance has been proven by experiments showing that pharmacologic or genetic suppression of inflammatory signaling improves insulin sensitivity.[20-22] The role of inflammatory pathways in NAFLD are also under active study, with available data indicating that inflammation plays an etiologic role in hepatic insulin resistance as well as hepatic steatosis and steatohepatitis.

Fat-Induced Inflammatory Signals in the Liver and Their Relation to NAFLD

Fatty acids[12,23,24] and high-fat feeding[9,21,25] can directly induce inflammatory signaling in the liver even in the absence of obesity or systemic insulin resistance.[9] Excess fat activates JNK and IKK in hepatocytes, which can induce hepatic insulin resistance, inflammatory cytokine expression, and in some instances cell death.[12,20,21,24,26,27] JNK and IKK both have the ability to stimulate the transcription of inflammatory target genes through their activation of activator protein-1 (AP-1) and NF-κB, respectively. Indeed, high-fat feeding in mice induces a hepatic profile of inflammatory gene expression closely mimicking that of mice expressing a hepatocyte-specific IKK transgene,[20,21,28] and conversely, mice with a

targeted disruption of either IKK or JNK are resistant to diet-induced insulin resistance and hepatic steatosis.[20,21,25,27,29] Activation of JNK and IKK in hepatocytes has broad consequences. Directly or indirectly, both kinases promote the expression of lipogenic genes within the liver.[25,28,30] Similarly, fat-related activation of IKK/NF-κB and JNK in hepatocytes stimulates the expression of cytokines and cell-adhesion molecules,[20,21,27,29] features that likely contribute to steatohepatitis. Indeed, in the methionine-choline-deficient (MCD) model of murine steatohepatitis, blockade of either IKK or the JNK-1 isoform of JNK significantly limits liver injury and inflammation.[27,31] Studies indicate that JNK is also an important mediator of "lipotoxicity" in the liver, based on its involvement in saturated fatty acid–induced apoptosis of hepatocytes in *in vivo* and *in vitro* mouse models.[12,27,32] Taken together, these myriad effects assign an important role to hepatocyte-derived IKK and JNK in inflammatory and cytotoxic pathways in obesity-related NAFLD.

In addition to activating inflammatory signaling directly in hepatocytes, fat stimulates innate immune processes locally within the liver that can result in organ damage. One is the up-regulation of the death receptor Fas, an alteration that correlates directly with disease severity in NAFLD.[26,33] Hepatic steatosis not only increases Fas expression by hepatocytes but also enhances the vulnerability of hepatocytes to Fas-mediated apoptosis[34,35] by perturbing cell-surface expression of the hepatocyte growth factor receptor cMet, a competitive inhibitor of Fas-Fas ligand (FasL) interactions.[35,36] Furthermore, fat-laden hepatocytes themselves express high levels of FasL,[35] creating an environment favoring hepatocellular suicide. The importance of Fas in the pathogenesis of murine NASH was recently demonstrated using a cMet peptide as a synthetic inhibitor of Fas-FasL interactions. This peptide reduced cell death and hepatic inflammation in both leptin-deficient and MCD-fed mice, and in MCD mice it even inhibited hepatic fibrosis.[35] These results point to cell death as a pivotal stimulus to NASH, echoing similar observations in other liver diseases such as viral hepatitis and obstructive cholestasis.[37,38]

TLRs, which are present on all resident cells in the liver,[39-43] act as innate immune sensors of foreign or abnormal structures. Select pattern recognition receptors figure prominently in the pathogenesis of NASH because of their potential for activation by saturated fatty acids[19] and because of their interaction with bacterial products such as Gram-negative endotoxin, which is found in the circulation of animals with obesity and fatty liver.[44,45] High-fat and MCD feeding induce hepatic expression of TLR2 and TLR4 as well as the TLR4 coreceptors CD14 and MD2.[21, 45] Steatosis also sensitizes the liver to challenge by TLR4 ligands.[45-47] Signaling through these receptors can promote NASH by inducing hepatic expression of a host of proinflammatory mediators.[43] In human beings, NAFLD has been associated with small intestinal bacterial overgrowth, although not necessarily through bacterial interactions with TLRs.[48] Likewise, the intestinal microbiome has recently been reported to play a central role in the development of obesity and fatty liver disease, not because of effects on innate immunity but instead because of direct effects on nutrient and energy metabolism.[49,50] Even so, probiotics, which reportedly suppress TLR-related responses by altering intestinal flora,[51,52] improve liver injury and inflammation in animals and humans with fatty livers,[53,54] and dietary n-3 polyunsaturated fatty acids, which are known inhibitors of TLR signaling,[55] suppress necroinflammation and fibrosis in experimental fatty liver disease.[56] The therapeutic success of these molecules suggests that the composition of the intestinal flora can influence innate immune processes leading to NAFLD.

Innate immune responses activated within fatty livers have great potential for amplification through cellular and humoral cross-talk. For example, hepatocyte apoptosis stimulates chemokine production[57] and induces Kupffer cells to produce FasL and cytokines,[58] which can augment cell death and promote hepatic inflammation. Hepatocytes, Kupffer cells, and

possibly other resident liver cells can also be stimulated to produce cytokines and chemokines through intracellular activation of IKK and JNK (see above) or extracellular activation of TLRs.[20,21,31,45] These compounds can then act in an autocrine or paracrine fashion to induce cell death, stimulate production of reactive oxygen species and recruit leukocytes from the circulation. A number of proinflammatory cytokines and chemokines (TNFα, interleukin-1 [IL-1], IL-6, IL-8, IL-12, IL-18, TNF, and macrophage inflammatory pro-tein-2) are up-regulated in fatty livers.[21,23,26,44,59-63] TNFα has been extensively studied as a putative mediator of NASH, albeit with mixed results.[31,53,59,64-66] More recently, attention has turned to the overall profile of proinflammatory cytokines in fatty livers, which is typical of that seen in T helper-1 (Th1) lymphocyte responses. Although NASH is not classically considered a Th1-polar-ized disease, data from several recent reports suggest that an imbalance resulting from a relative excess in proinflammatory Th1 cytokines such as interferon-γ and a relative deficiency of anti-inflammatory cytokines such as IL-4 and IL-10 can influence fatty liver disease.[60,67] Natural killer T (NKT) cells are regulatory T lymphocytes that are activated by specific glycolipids presented by the major histocompatibility class I–like molecule CD1d. T cells are typically considered components of adaptive immunity, but NKT cells are preactivated *in situ* by endogenous (self) glycolipids and are considered to be innate immune effectors.[68] They likely play a role in the innate or intrinsic propensity of an individual to mount either Th1 or Th2 cytokine responses. Th2-skewed IL-4–producing NKT cells are diminished in number in mice with leptin-defi-ciency[60] or diet-induced obesity.[61] The severity of liver injury in these animals is inversely proportional to NKT cell number. The reason for this reduction appears multifactorial, involving activation-induced death of NKT cells in response to Kupffer cell–derived IL-12[61] as well as decreased NKT survival due to reduced exposure to CD1d on the hepatocyte surface.[69] Notably, adoptive transfer of IL-4–producing NKT cells results in amelioration of steatosis and diminished hepatic levels of the Th1-like cytokine IL-12 in Kupffer cells.[67,70] These experiments suggest that NKT cell– derived cytokines such as IL-4 play a protective role in diet-induced NAFLD, and they intimate that polarized Th1 cytokines play pathophysiologic roles in fat-induced inflammation and steatosis.

Inflammatory Signals in Adipose Tissue and Their Relation to NAFLD

Although the metabolic syndromes of atherosclerosis and NAFLD are clearly associated with organ-specific inflammation, the discovery that adipose tissue itself is inflamed in obesity was not made until 2003.[71] At that time, macrophages were identified as the principal effectors of adipose tissue inflammation, based on microarray studies showing that obese mice exhibit markedly increased expression of macrophage-specific genes in white adipose tissue, combined with immunohistochemical studies demonstrating significant and selective infiltration of the adipose tissue by macrophages.[72-74] This breakthrough is relevant to NAFLD, because emerging data suggest that the inflammatory state of adipose tissue controls lipid homeostasis in other organs, including the liver.[3,4,75] The mechanisms by which obesity causes macrophages to infiltrate adipose tissue are currently unknown. Some suggest that excessive lipid loading causes adipocytes to undergo necrosis, which activates resident macrophages and promotes macrophage recruitment from the circulation (Fig. 2A).[76] Others argue that obesity activates macrophages in the circulation[77] and that these cells are then recruited to adipose tissue by an obesity-related signal emanating from adipocytes (Fig. 2B). In this regard, high-fat feeding induces adipocytes to express the chemokine monocyte chemoattractant protein-1 (MCP-1), whose binding partner is C-C chemokine receptor-2 (CCR2).[3,4] Adipocyte-derived MCP-1 stimulates the recruitment of CCR2-expressing macrophages into adipose tissue.[3,4] Importantly, it also causes hepatic insulin resistance and hepatic steatosis, and even affects behavior, enhancing food intake. Conversely, mice deficient in either MCP-1 or CCR2 do not exhibit macrophage infiltration into adipose tissue and are protected from diet-induced hepatic steatosis and insulin

resistance.[3,4] Blockade of the MCP-1/CCR2 axis reduces, but does not eliminate, high-fat diet–induced infiltration of macrophages into adipose tissue, raising the possibility that other inflammatory and chemotactic agents also contribute to this process. One candidate is C-X-C chemokine ligand-14 (CXCL14), which is selectively induced in adipose tissue of obese mice.[78,79] Like MCP-1–deficient mice, CXCL14-defi-cient mice exhibit diminished adipose tissue macrophage recruitment, improved insulin responsiveness, and reduced liver weight in comparison to wild-type mice in response to high-fat feeding.[78] Macrophage recruitment to adipose tissue may also involve the proinflammatory cytokine osteopontin.[80] Evidence for this comes from osteopontin-deficient mice, which exhibit impaired macrophage recruitment into adipose tissue upon high-fat feeding as well as attenuated systemic inflammation and improved insulin resistance.[81]

Additional studies demonstrate that obesity affects not only macrophage recruitment to, but also macrophage phenotype within, adipose tissue. Plasticity and functional polarization are characteristics of macrophages; these cells can be induced to express features typical of either inflammatory or noninflammatory (resident) macrophages, depending on their exposure to divergent stimuli. Macrophage phenotype has been defined across at least two separate polarization states, termed M1 and M2.[82] M1 or “classically activated” macrophages are induced by proinflammatory mediators such as lipopolysaccharide and interferon-γ. They are characterized by a high capacity to present antigen, robust IL-12, IL-6, TNF-α and IL-23 production,[83] and consequent activation of polarized Th1 responses. M1 macrophages also produce reactive oxygen species such as nitric oxide (NO) via activation of inducible nitric oxide synthase (iNOS). The term M2 or “alternatively activated” macrophages has been applied to macrophages generated in response to IL-4 and IL-13, which can promote Th2 responses.[82] These cells have low proinflammatory cytokine expression, and instead express high levels of the anti-inflammatory cytokine IL-10 and the IL-1 decoy receptor. Another property of M2 macrophages is that they have elevated levels of arginase, which competes with iNOS for the substrate L-arginine. Whereas iNOS utilizes L-arginine to generate reactive NO species with microbicidal and proinflammatory M1 effects, arginase hydrolyzes arginine and promotes anti-inflammatory M2 effects.[84, 85] Recently published data revealed that adipose tissue macrophages from lean mice express many genes characteristic of M2 or “alternatively activated” cells, whereas macrophages from mice with diet-induced obesity express fewer M2 genes and more genes such as TNFα and iNOS, which are characteristic of the M1 phenotype.[86]

A further link between M1 polarization of macrophages and NAFLD comes from studies of the peroxisome proliferator-activated receptor-γ (PPARγ). PPARγ, a genetic sensor of unsaturated fatty acids, serves as a ligand for the RXR nuclear receptor where it classically regulates processes related to fatty acid and glucose metabolism. In addition, PPARγ has a profound influence on inflammatory responses in macrophages. PPARγ agonists inhibit macrophage cytokine production by antagonizing the activity of the proinflammatory transcription factors AP-1, signal transducer and activator of transcription (STAT), and NF-κB.[87, 88] One recent report indicated that PPARγ is also required for the complete polarization of macrophages toward the noninflammatory, reparative M2 phenotype.[89] Moreover, pharmacologic PPARγ agonists are able to convert inflammatory M1 macrophages to “alternatively activated” noninflammatory M2 macrophages.[90] Together these data indicate that PPARγ agonists play a key role in the regulation of macrophage phenotype and function. In the context of NASH, this information offers a potentially unique explanation for the therapeutic benefit of PPARγ agonists (thiazolidinediones).[91-93] It suggests that the immunomodulatory capabilities of these agents are paramount to their efficacy in controlling the inflammatory and perhaps even the metabolic abnormalities that accompany NASH. In support of this theory, mice lacking PPARγ in macrophages exhibit enhanced activation of inflammatory signals in the liver at baseline and develop pronounced

hepatic insulin resistance in response to high-fat feeding.[94] Moreover, patients with insulin resistance who are treated with rosiglitazone show reduced parameters of inflammation even before they exhibit any improvement in insulin sensitivity.[95] Also pertinent is that IL-4, a Th2 cytokine produced by NKT cells, is a potent inducer of endogenous PPARγ ligands.[96] This raises the intriguing possibility that in the liver, NKT cells suppress the Th1/M1 environment characteristic of NAFLD through IL-4–mediated production of anti-inflammatory PPARγ agonists.

When viewed in aggregate, these new and compelling findings suggest that obesity-associated steatohepatitis may be more closely linked to adipose tissue macrophage activation than to the metabolic effects of excess fat stores. Indeed, obesity-induced NAFLD goes hand in hand with adipose tissue macrophage activation, but not always with adiposity, as shown in MCP-1–deficient or CCR2-defi-cient mice that are protected from diet-induced hepatic steatosis even though they still become obese. Collectively, these studies support a paradigm in which adipose tissue macrophages play a major role in the systemic metabolic syndromes of insulin resistance and NAFLD. For the moment, the exact role of adipose tissue macrophages in the pathogenesis of NASH remains uncertain, because investigators focusing on inflammatory adipose tissue have not uniformly extended their work to include a careful examination of liver injury or inflammation. Still, the available data indicate that M1-polarized inflammatory adipose tissue macrophages are central to the development of obesity-associated hepatic steatosis and insulin resistance.[3,4] Macrophage infiltration of adipose tissue promotes hepatic steatosis and insulin resistance even in the absence of any evidence of simultaneous macrophage influx into the liver.[3,73] This suggests that at least some features of NAFLD, and perhaps even NASH, arise through endocrine interactions with fat. It is possible that classically activated M1 macrophages or inflamed adipocytes secrete a systemic inflammatory mediator that triggers inflammation at remote sites. Some have touted TNFα as such a factor, given its landmark association with insulin resistance,[97] but it is noteworthy that TNF receptor 1–deficient mice can still develop diet-induced NASH.[31,59] Alternatively, adipose tissue-derived MCP-1 itself, which circulates at high levels in obese animals, could serve as an endocrine mediator of NAFLD through an indirect mechanism, because hepatocytes are not known to express CCR2. It is also possible that instead of stimulating the synthesis of a proinflammatory factor, inflamed macrophages within adipose tissue inhibit the elaboration of a systemic anti-inflammatory mediator from adipocytes. A leading candidate molecule is adiponectin, which is normally secreted by lean, but not obese, adipose tissue.[98,99] Adiponectin appears to have anti-inflammatory properties and is sharply lowered in the serum of obese mice. Intriguingly, adiponectin is present in high levels in obese CCR2-deficient mice, which have neither inflamed adipose tissue nor hepatic steatosis.[4] The importance of adiponectin to the development of diet-induced metabolic changes was highlighted by a recent study in which leptin-deficient obese mice were genetically engineered to produce high levels of adiponectin.[75] Adiponectin overexpression resulted in a reduction in adipose tissue macrophage infiltration and a reduction of circulating IL-6 and TNFα, despite a massive expansion of adipose tissue fat. In addition, adipose tissue macrophages from obese adiponectin transgenic mice possessed an alternatively activated M2 phenotype, rather than the typical obesity-induced M1 macrophage phenotype. These data underscore the powerful anti-inflammatory function of adiponectin, which may underlie its protective effects against hepatic steatosis.[100] Yet another means by which adipose tissue could contribute to an inflammatory phenotype in liver is for macrophages to become activated within fat and then traffic to the liver, triggering inflammation. Although there are no data to support this notion, peripheral blood macrophages in obese mice appear to be skewed toward the M1 phenotype,[77] and resident liver macrophages (Kupffer cells) have an inflammatory phenotype with a predominant expression of the M1 cytokines TNFα and IL-12.[60,61]

Conclusion

As research into the pathophysiology of NAFLD expands, it is becoming clear that the disease involves a number of innate immune processes both within and outside the liver. It is also becoming evident that steatosis and inflammation actively influence each other by multiple mechanisms, even across organs, as shown in the case of inflamed adipose tissue affecting the metabolic status of the liver. These findings support a theme already common among those who research the metabolic syndrome—that the distinction between metabolism and inflammation has blurred.[1,15,71,101] Hotamisligil[1] recently noted that the close interconnection between metabolism and inflammation creates a “chicken and egg” dilemma in which it is difficult to tell which process (nutrient excess or inflammation) actually initiates the metabolic syndrome. The available data support a model in which lipids are the primary stimulus to innate immune activation, with the resulting inflammatory milieu then causing metabolic dysregulation (insulin resistance and further fat deposition) and setting into motion a vicious cycle that culminates in end-organ dysfunction.

With regard to innate immunity in NAFLD, yet to be reconciled is whether fatty liver disease is an organ-autonomous process or absolutely requires a contribution from inflamed adipose tissue. Although some studies suggest that features of NAFLD are inducible by events occurring solely within the liver,[9,20] others clearly show that inflamed fat worsens these abnormalities.[3,4] Still others argue that NASH occurs in patients with lipodystrophy, who have no adipose tissue and thus no adipose tissue inflammation.[102] Although the last observation would seem to support a liver-autonomous view of NAFLD pathogenesis, it is important to recall that normal adipose tissue produces adiponectin and other adipokines whose functions are to promote lipid homeostasis and suppress inflammation.[103] Metabolic and immune interplay between liver and adipose tissue, therefore, is likely operative in both health and disease, and both organs must be taken into consideration to obtain a complete and accurate picture of NASH pathogenesis.

From a translational perspective, research on innate immune activation in NAFLD has led to several diagnostic and therapeutic advances. For example, high serum levels of MCP-1 and low serum levels of adiponectin are being exploited as markers of disease severity.[93,104,105] Similarly, hepatocyte apoptosis in fatty livers, much of which is likely Fas-mediated, forms the basis for using cytokeratin-18 as an independent serum marker of NAFLD.[106,107] One rationale for using fish oil to prevent or treat NASH comes from scientific evidence that n-3 polyunsaturated fatty acids suppress proinflammatory signaling through TLRs.[108] Furthermore, the anti-inflammatory properties of PPARγ agonists offer an explanation for their efficacy in NASH in spite of persistent adiposity.[109] As understanding of the complex relationship between metabolism and inflammation grows, there will undoubtedly be more opportunities to apply knowledge of innate immunity to the management and ultimately the prevention of fatty liver disease.

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Abbreviations

CCR2	C-C chemokine receptor-2
ER	endoplasmic reticulum
IKK	inhibitor of NF-κB kinase
IL	interleukin
iNOS	inducible nitric oxide synthase
IRS	insulin receptor substrate
JNK	Jun N-terminal kinase
MCD	methionine-choline-deficient

MCP-1	monocyte chemoattractant protein-1
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NF-κB	nuclear factor κB
NKT	natural killer T cell
PKC	protein kinase C
PPARγ	peroxisome proliferator-activated receptor-γ
TLR	Toll-like receptor

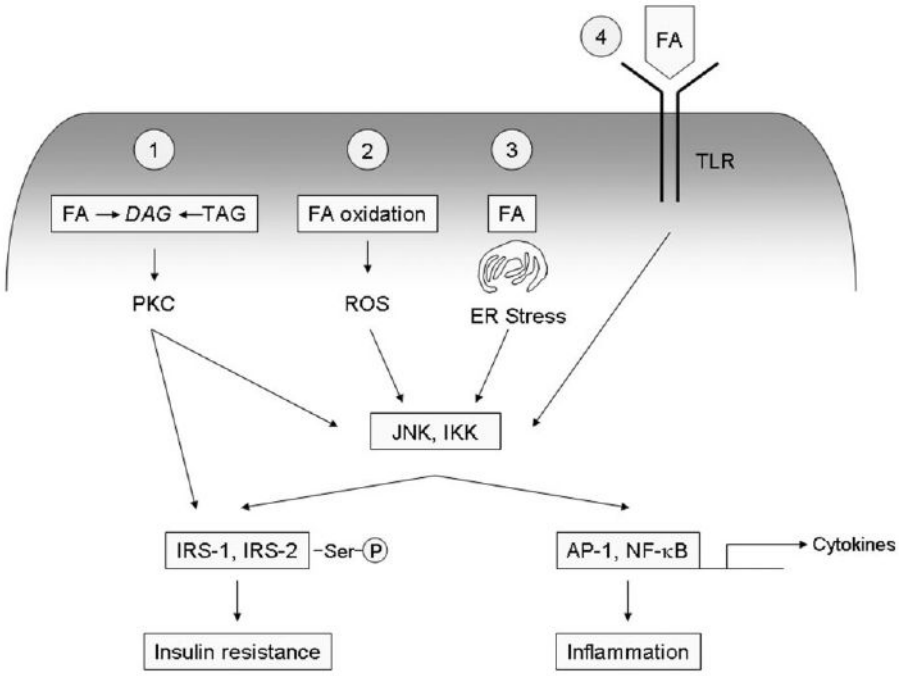

Fig. 1.

Activation of inflammatory signaling pathways by fat. Excess fat or fatty acids (FA) can activate a number of intracellular signaling pathways that lead to inflammation through IKK and JNK IKK and JNK cause inflammation by promoting formation of the transcription factors AP-1 and NF-κB, which activate transcription of a host of proinflammatory genes including cytokines, chemokines, and cell adhesion molecules. PKC, IKK and JNK can also cause insulin resistance by promoting aberrant serine phosphorylation of IRS-1 and IRS-2. (1) The lipid intermediate diacylglycerol (DAG), formed during the synthesis or hydrolysis of triacylglycerol (TAG), can activate PKC, which causes downstream activation of IKK and JNK. (2) Fatty acid oxidation yields reactive oxygen species (ROS), which can directly activate IKK and JNK. (3) Excess fat can promote ER stress, which activates IKK and JNK through the intermediate kinases IRE-1 and PERK. (4) Extracellular fatty acids can act as ligands for TLR, which signal through IKK and JNK.

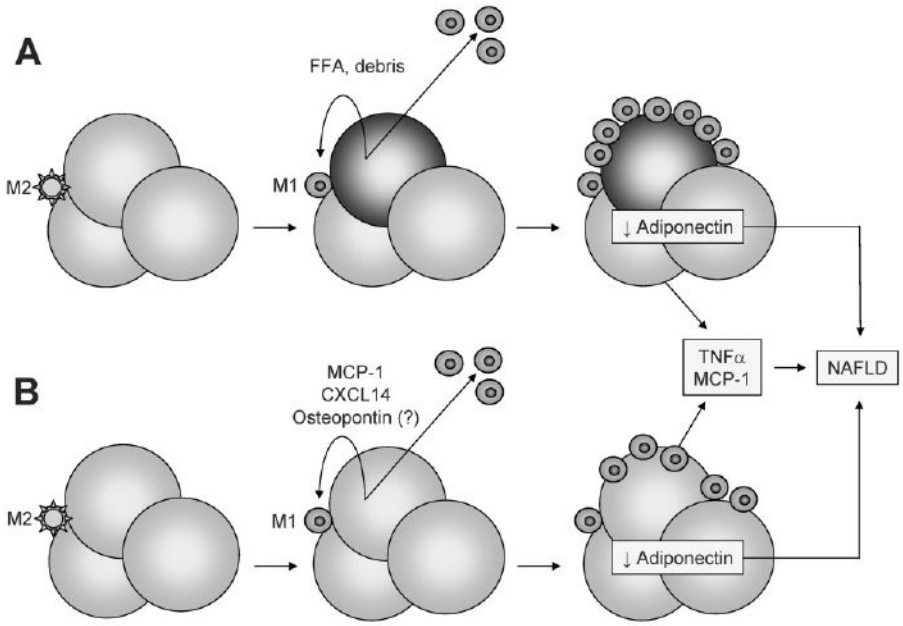

Fig. 2.

Proposed mechanisms of adipose tissue macrophage activation and their contribution to NAFLD. (A) Normal adipose tissue contains a small number of resident macrophages with an M2 (anti-inflammatory) phenotype. Expansion of adipocytes with fat in obesity can provoke adipocyte necrosis, with the released cellular debris and free fatty acids (FFA) activating resident macrophages and signaling the recruitment of M1 (proinflammatory) macrophages from the circulation. The resulting inflamed fat produces high levels of TNFa and MCP-1 and low levels of adiponectin, which can contribute to NAFLD. TNFa and MCP-1 can derive from both adipocytes and macrophages, whereas adiponectin is produced exclusively by adipocytes. (B) Obese adipocytes remain viable but are induced to secrete MCP-1, CXCL14, and perhaps osteopontin. This attracts and activates macrophages to an M1 phenotype. The end result is the same, with inflamed fat producing high levels of TNFa and MCP-1 and low levels of adiponectin.