Molecular and cellular mechanisms linking inflammation to insulin resistance and β-cell dysfunction

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Obesity is a major public health problem worldwide, and it is associated with an increased risk of developing type 2 diabetes. It is now commonly accepted that chronic inflammation associated with obesity induces insulin resistance and β-cell dysfunction in diabetic patients. Obesity-associated inflammation is characterized by increased abundance of macrophages and enhanced production of inflammatory cytokines in adipose tissue. Adipose tissue macrophages are suggested to be the major source of local and systemic inflammatory mediators such as tumor necrosis factor α, interleukin (IL)-1β, and IL-6. These cytokines induce insulin resistance in insulin target tissues by activating the suppressors of cytokine signaling proteins, several kinases such as c-Jun N-terminal kinase, IκB kinase β, and protein kinase C, inducible nitric oxide synthase, extracellular signal-regulated kinase, and protein tyrosine phosphatases such as protein tyrosine phosphatase 1B. These activated factors impair the insulin signaling at the insulin receptor and the insulin receptor substrates levels. The same process most likely occurs in the pancreas as it contains a pool of tissue-resident macrophages. High concentrations of glucose or palmitate via the chemokine production promote further immune cell migration and infiltration into the islets. These events ultimately induce inflammatory responses leading to the apoptosis of the pancreatic β cells. In this review, the cellular and molecular players that participate in the regulation of obesity-induced inflammation are discussed, with particular attention being placed on the roles of the molecular players linking inflammation to insulin resistance and β-cell dysfunction. (Translational Research 2016;167:228–256)

Abbreviations: AMPK = AMP-activated protein kinase; ATM = adipose tissue macrophages; CCL = chemokine (C-C motif) ligand; CCR2 = chemokine (C-C motif) receptor 2; CX3CL1 = chemokine (C-X3-C motif) ligand 1; CX3CR1 = chemokine (C-X3-C motif) receptor; CXCL = CXC chemokine ligand; DAG = diacylglycerol; ER = endoplasmic reticulum; ERK = extracellular signal-regulated kinase; FFA = free fatty acid; GLUT = glucose transporter; HFD = high-fat diet; IAPP = islet amyloid polypeptide; IFN-γ = interferon gamma; IKKβ = IκB kinase β; IL-6 = interleukin 6; IL-1β = interleukin 1 β; IL-10 = interleukin 10; IL-4 = interleukin 4; IL-13 = interleukin 13; iNOS = inducible nitric oxide synthase; IRE1 = inositol-requiring protein 1; IRS = insulin receptor substrate; JNK = c-Jun N-terminal kinase; LAR = leukocyte antigen related; LPS = lipopolysaccharide; LTB4 = leukotriene B4; MAPK = mitogen-activated protein kinase; MCP-1 = monocyte chemotactic protein 1; mTORC = mammalian target of rapamycin complex; MyD88 = myeloid differentiation primary response gene 88; NF-κB = nuclear factor kappa B; NK cell = natural killer cells;

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NLRs = NOD-like receptors; NLRP3 = NLR pyrin domain containing 3; NO = nitric oxide; PAMPs = pathogen-associated molecular patterns; PDX-1 = pancreatic and duodenal homeobox 1; PI3-kinase = phosphoinositide (PI) 3-kinase; PKC = protein kinase C; PP2A = protein phosphatase 2A; PRRs = pattern recognition receptors; PTEN = phosphatase and tensin homolog; PTPs = protein tyrosine phosphatases; PTP1B = protein tyrosine phosphatase 1B; ROS = reactive oxygen species; SHIP2 = SH2 domain-containing inositol-5-phosphatase 2; SOCS = suppressors of cytokine signaling; T2D = type 2 diabetes; Th1 = T-helper 1; TLR4 = toll-like receptor 4; TNF-α = tumor necrosis factor α; TRAF2 = TNF receptor–associated factor 2; Treg cell = regulatory T cell; TSC1 = tuberous sclerosis 1; TSC2 = tuberous sclerosis 2; UPR = unfolded protein response; WAT = white adipose tissue

INTRODUCTION

The rapidly increasing prevalence of type 2 diabetes (T2D) worldwide is one of the most serious and challenging health problems in the 21st century. The World Health Organization stated that 347 million people worldwide were suffering from T2D in 2008 and this number is estimated to be doubled by 2030.[1] T2D that accounts for ~90% of all cases of diabetes is characterized by insulin resistance and β-cell dysfunction. Insulin resistance is a pathologic state resulting from the inability of the peripheral target tissues (skeletal muscle, liver, and adipose) to respond effectively to normal circulating concentrations of insulin. β-Cell dysfunction is a condition when β cells fail to produce sufficient insulin.[2] T2D is reaching an epidemic stage with increasing the prevalence of obese people.[3] The development of T2D results from an interaction of a patient's genetic background with social and environmental factors[4] (Fig 1). Disorders of the nervous and endocrine systems, a sedentary lifestyle, psychological stresses, a high-fat diet (HFD), overeating, age, smoking, alcohol intake, and inadequate sleep are some of the causes that can contribute to obesity.

Inflammatory pathways have been suggested as the underlying mediators of obesity-induced T2D.[5] Obesity is associated with a state of chronic, low-grade inflammation. This low-grade inflammation is characterized by higher levels of circulating proinflammatory cytokines and free fatty acids (FFAs). These factors can then interfere with normal insulin function and secretion and thereby induce insulin resistance and β-cell dysfunction. In this review, the recent evidence linking low-grade chronic inflammation to T2D is summarized. First, the cellular and molecular mechanisms leading to low-grade inflammation in obesity are discussed. Second, the role of inflammation in insulin resistance and β-cell dysfunction is explored.

OBESITY

The prevalence of obesity worldwide has increased at alarming rates in past few decades. Consequently, obesity and associated disorders are now a serious threat for public health.[6] According to the new analysis from the Global Burden of Disease Study 2013, there has been a startling increase in the rates of obesity and overweight in both adults (28% increase) and children (up by 47%) in the past 33 years, with the number of overweight and obese people rising from 857 million in 1980 to 2.1 billion in 2013.[7]

INFLAMMATION

In February 2004, the magazine Time dedicated its cover to inflammation and named it "The Secret Killer." During the past decade, it became clear that inflammation has a central role in the pathogenesis of obesity and T2D.[8] Before focusing on the role of inflammation in insulin resistance and β-cell dysfunction, it would be helpful to introduce this term and a few crucial concepts that might differ from the classical context that have already been used.

In the classic literature, inflammation is the immune response of tissues invoked to cope with injuries manifesting as swelling, redness, pain, and fever.[9] This is a short-term process that contributes to tissue repair and involves the integration of many complex signals in distinct cells and organs. However, the long-term consequences of a prolonged inflammation have detrimental effects, and it is now well understood that its dysregulation has as major role in the pathogenesis of many diseases such as T2D, rheumatoid arthritis, atherosclerosis, asthma, and other autoimmune diseases. This condition is principally triggered by excess nutrients and engages a similar set of molecules and signaling pathways to those involved in the classical inflammation.

IS OBESITY-INDUCED T2D AN INFLAMMATORY DISEASE?

Hotamisligil et al[10] made an early connection between obesity, inflammation, and T2D, by reporting the induction of tumor necrosis factor (TNF)-α, a proinflammatory cytokine, in adipose tissue from 4 rodent models of obesity and T2D. They demonstrated that the neutralization of TNF-α in obese fa/fa rats

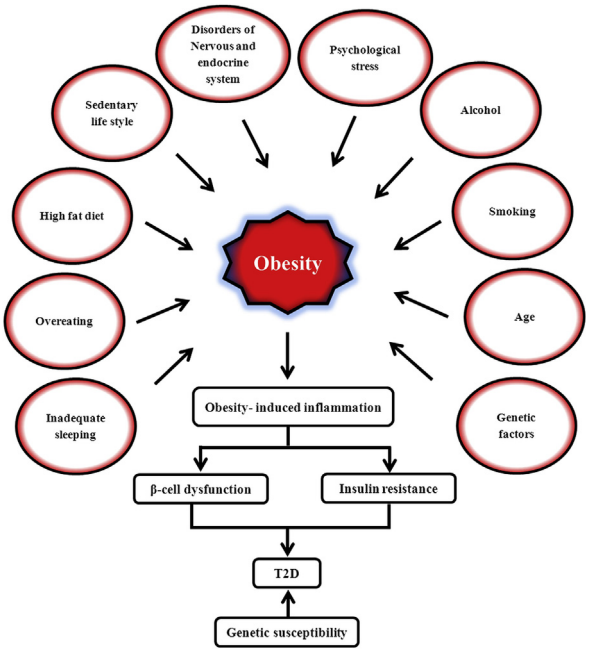

Fig 1. Impact of genetic background, behavioral factors, and environmental triggers on the development of T2D. Genetic susceptibility, behavioral (eg, sedentary lifestyle, high-fat diet), and environmental factors (eg, psychological stress) have complementary effects on the development of T2D. T2D, type 2 diabetes.

ameliorated insulin resistance. Furthermore, mice deficient in TNF-α had significantly improved insulin sensitivity in both the diet-induced and the ob/ob models of obesity.[11] From the time of this finding, many studies have shown that the inflammatory signals disrupt the insulin action and mediate insulin resistance in obesity. Similar to the case of rodents, in obese individuals excess adipose mass has been associated with increased levels of the proinflammatory marker C-reactive protein in the blood.[12] Increased levels of C-reactive protein, its inducer interleukin 6 (IL-6), and IL-1β were predictive of the development of T2D in various populations.[12] In both murine models and humans, it is evident that obesity triggers the accumulation of immune cells, particularly macrophages, in visceral adipose, liver, skeletal muscle, and pancreas tissues resulting in a local and systemic inflammation that ultimately leads to systemic insulin resistance and β-cell dysfunction.

ADIPOSE TISSUE AS A SOURCE OF INFLAMMATION IN T2D

The link between obesity and inflammation seems to be the adipose tissue itself. Adipose tissue is not only considered a lipid storage organ, but also a critical site in the generation of inflammatory responses and mediators. Recent studies have documented that there are several connections indeed between adipose tissue and the immune system. For a start, a diverse set of immune cells (including T cells, macrophages, and dendritic cells) can be normally found in the adipose tissue.[13] Moreover, it has been suggested that white adipocytes share embryonic origin with the immune cells, and characterization of adipose tissue-resident lymphocytes led to the notion that this tissue was an ancestral immune organ.[13] In addition, the presence of immature hematopoietic cells in adipose tissue proposed that this tissue may be a site for formation and maturation of immune cell precursors.[14]

On the other hand, the adipose tissue can influence and communicate with the liver, muscle, pancreas, and other organs through the release of cytokines, adipokines, and FFAs. Tissue-specific knockout mice have provided many unique insights into the significance of the signaling pathways involved in these processes. Genetic modifications of the adipocytes that improve adipocyte insulin sensitivity lead to systemic insulin sensitivity, with enhanced insulin actions in the liver and skeletal muscle cells. Thus, adipose tissue is often referred to as the master regulator in the development of systemic insulin resistance and a potential window through which we can explore the link between obesity and inflammatory responses.

The adipose tissue is classified in 2 major depots: white adipose tissue (WAT) and brown adipose tissue. Brown adipose tissue is implicated in cold- and diet-induced thermogenesis (nonshivering thermogenesis), modulation of body temperature, and energy expenditure.[15] WAT is specialized in the storage of energy in the form of triacylglycerol and protects other organs and tissues from the ectopic fat accumulation and consequently from lipotoxicity.[15] Until the 1990s, the WAT functions were only associated with passive energy storage, thermal insulation, and organ protection from mechanical damage, whereas it is now recognized as a dynamic and heterogeneous endocrine organ. It is composed of connective tissue, an extracellular matrix, and different cell types such as preadipocytes, adipocytes, adipose tissue macrophages (ATMs), fibroblasts, endothelial cells, and stem cells.[16] Adipose tissue secretes numerous bioactive peptides collectively known as adipokines.[17] These adipokines include the peptides involved in glucose homeostasis such as adiponectin, resistin, apelin, and visfatin and hormones involved in energy homeostasis such as leptin. Adipose tissue also produces chemokines such as monocyte chemotactic protein 1 (MCP-1) and IL-8, proinflammatory cytokines such as IL-6, IL-1, angiotensin II, and TNF-α, and anti-inflammatory cytokines such as IL-10. Thus, it is now well accepted that adipose tissue as an endocrine system plays a pivotal role in regulating the energy balance, glucose homeostasis, and immune system function.

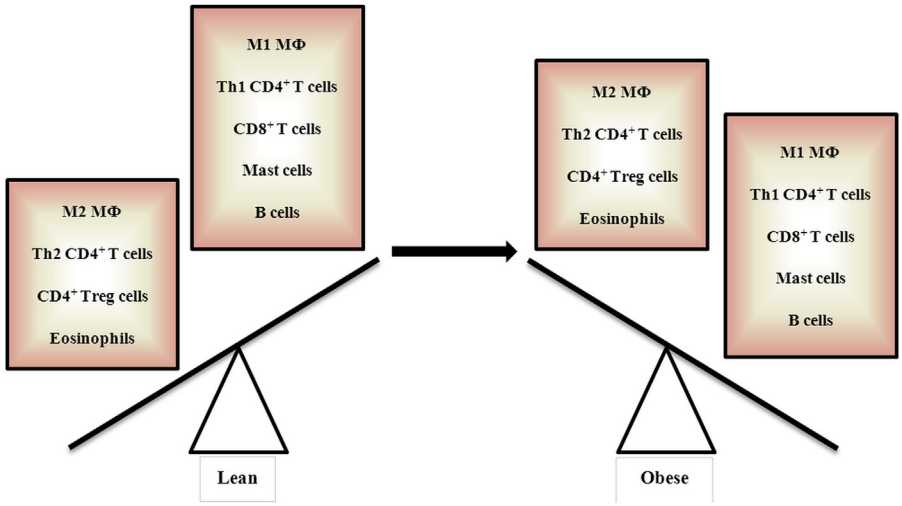

Fig 2. Changes in immune cell populations in adipose tissue in obesity. Lean adipose tissue contains a greater proportion of M2/M1 macrophages. It also contains a large number of regulatory T cells (Treg cells). Obesity and adipocyte hypertrophy leads to adipocyte necrosis and an increase in proinflammatory M1 macrophage numbers. There is also a reduction in Treg cells and an increase in B cells, $CD4^+$ T-helper 1 cells (Th1), and $CD8^+$ T cells in adipose tissues of obese subjects.

Excessive growth of adipose tissue leads to adipocyte hypertrophy and a disturbance in adipokines secretory profiles. This has been primarily attributed to an imbalance between the secretions of proinflammatory and anti-inflammatory adipokines.[8] Adipocytes[18] as well as preadipocytes, macrophages, and adipose stem cells contribute to the production of proinflammatory cytokines in obesity.[19] Major immune cell populations that play key roles in the inflammatory responses in obesity are illustrated in Fig 2.

MACROPHAGES PLAY A CENTRAL ROLE IN OBESITY-INDUCED INFLAMMATION

An important finding that unveiled the cause of tissue inflammation was infiltration of adipose tissue with large numbers of macrophages.[20] These ATMs form approximately 40% of the cells in obese adipose tissue.[20] Extensive studies have shown that the ATMs have a key role in systemic insulin resistance, glucose tolerance, and T2D.[17] In obesity, the proinflammatory pathways in ATMs are highly activated and this activation leads to the secretion of a variety of cytokines, such as TNF-α and IL-1β.[17] These cytokines can then act through a paracrine manner, or they can leak into the systemic circulation, causing a decrease in insulin sensitivity in the insulin target cells (adipocytes, hepatocytes, and myocytes) and β-cell dysfunction in the pancreas.

Macrophage heterogeneity. Macrophages have been classified into 2 groups: the classically activated macrophages, termed M1, and alternatively activated macrophages, termed M2. M1 can be induced in vitro by growing the bone marrow–derived hematopoietic cells with granulocyte-macrophage colony–stimulating factor. M2 can be induced by culturing the bone marrow–derived cells with macrophage colony–stimulating factor and IL-4. The cytokine profile of M1 macrophages is proinflammatory and includes TNF-α, IL-6, and IL-1. On the contrary, M2 macrophages express anti-inflammatory factors such as IL-10, transforming growth factor β, IL-1 receptor antagonist (IL-1RA)-α, IL-4, and arginase.[21] M1 macrophages are stimulated by interferon gamma (IFN-γ) and lipopolysaccharide (LPS), whereas M2 macrophages are activated by IL-4 and IL-13.[21]

Obesity reduces anti-inflammatory IL-10 production and increases the proinflammatory TNF-α production by inducing an M2 to M1 shift in ATM populations.[22] This increase in M1 ATM is because of either a "phenotypic switch" from M2 to M1 or additional recruitment of M1 macrophages from blood vessels. Lipotoxicity of the macrophages appears to be the major contributor for this phenotypic switch of M2 to M1.[23] Detailed mechanisms of the M2 to M1 switch have been previously reviewed by Olefsky and Glass.[17] Briefly, the toll-like receptor 4 (TLR4) ligands such as saturated FFAs activate nuclear factor kappa B (NF-κB) and activator protein 1 (AP1) transcription factors, and these activations lead to an increased production of proinflammatory cytokines such as TNF-α, IL-6, and IL-1 giving rise to the M1 phenotype. In the lean adipose tissue, this process is prevented by repression of TLR4-responsive genes by nuclear receptor corepressor complex.

Peroxisome proliferator–activated receptor γ along with IL-4 and IL-13 prevents the signal-dependent turnover of nuclear receptor corepressor and thus helps to maintain the M2 phenotype.

Recruiting macrophages into adipose tissue. The recruitment of macrophages into adipose tissue is the initial event in obesity-induced inflammation. Hypertrophic adipocytes secrete chemokines such as MCP-1, leukotriene B4 (LTB4), and others, providing a chemotactic gradient that attracts monocytes into adipose tissue. Once the monocytes migrate into the adipose tissue, they can differentiate into ATMs. After migration into adipose tissue, the proinflammatory ATMs also secrete their own chemokines, attracting additional macrophages and setting up a feed-forward inflammatory process.[17]

MCP-1 that is secreted by enlarging adipocytes[24] appears to be the key mediator of the initiation of adipose tissue inflammation in obesity. It has been shown that MCP-1 plays an important role in the recruitment of macrophages into the adipose tissue. MCP-1 binds to the chemokine (C-C motif) receptor 2 (CCR2) on macrophages to stimulate macrophage migration.[25] Weisberg et al[26] reported that the deletion of macrophage CCR2 or adipose tissue MCP-1 decreases the ATM content, reduces tissue markers of inflammation, and ameliorates insulin resistance. However, the opposite findings have been reported from the other studies. According to the study by Chen et al,[27] CCR2 and MCP-1 alone do not significantly affect the macrophage recruitment and this issue remains to be fully resolved.

Several other chemokines have also been implicated in the recruitment of inflammatory cells. LTB4, a potent chemotactic factor on neutrophils, is produced by adipocytes, where it can contribute to ATM infiltration. Indeed, it has been demonstrated in recent studies that knocking out the gene encoding the LTB4 receptor (BLT1) can protect mice from obesity-induced inflammation and insulin resistance.[28] Fractalkine also known as chemokine (C-X3-C motif) ligand 1 (CX3CL1) is expressed in adipocytes and macrophages and is markedly upregulated in obese human adipose tissue[29] and contributes to the adhesion of monocytes to adipocytes.[30] Although the CX3CL1-CX3CR1 (fractalkine receptor) system plays an important role in chronic inflammatory diseases such as atherosclerosis,[31] its role in adipose tissue inflammation is unknown.

Another chemoattractant factor that plays a central role in macrophage recruitment in adipose tissue is saturated FFAs leaked from the hypertrophied adipose tissue. In one study, Takahashi et al[32] showed that the supplementation with palmitate caused the recruitment of monocytes into hypertrophied murine adipocytes by inducing the MCP-1 production via the c-Jun N-terminal kinase (JNK) and NF-κB activation.

Lymphocytes. Besides macrophages, lymphocytes seem to be strongly implicated in inflammatory processes linked to obesity. T cells in adipose tissue are believed to play a part in obesity-induced inflammation by modifying the ATM numbers and their activation state.[33] T cells can be divided into 2 main classes according to the surface proteins. Cytotoxic $CD8^+$ cells express glycoprotein $CD8^+$, and $CD4^+$ cells express $CD4^+$ on their surface. $CD4^+$ T cells can be divided further into 2 lineages: T-helper 1 (Th1) cells that produce proinflammatory cytokines, and T-helper 2 (Th2) cells that produce anti-inflammatory cytokines.[34] Another $CD4^+$ lineage, regulatory T cells (Treg cells), can secrete anti-inflammatory signals, inhibit macrophage migration, and induce M2-like macrophage differentiation. The number of adipose tissue Treg cells decreases with obesity,[35] and an increase in the number of these cells in obese mice can improve insulin sensitivity.[35] It has been shown that $CD4^+$ T lymphocytes have a protective role against systemic insulin resistance, as the recombination-activating gene 1–deficient mice, which lack the T lymphocytes, developed a profound degree of insulin resistance when fed an HFD.[36] $CD8^+$ T cells, referred to as effector or cytotoxic T cells, also secrete proinflammatory cytokines. Nishimura et al[33] showed that the $CD8^+$ T cells are increased in obese adipose tissue, and they promote the recruitment and activation of ATMs with a concurrent reduction in $CD4^+$ helper and Treg cells.

The temporal pattern of accumulation of different T cells and macrophages in adipose tissue during the development of obesity is still not fully understood. Nishimura et al[33] proposed that the adipose tissue Th1 cells may initiate an inflammatory cascade before ATM infiltration. However, Strissel et al[37] recently found that the number of Th1 cells did not increase until 20 weeks after introduction to HFD, several months after the increase in ATMs and insulin resistance. Whatever the time course of inflammatory cell recruitment, although T cells clearly have a role in the development of inflammation and insulin resistance in vivo, they are not absolutely essential to the process, as obese mice depleted of lymphocytes can still mount an ATM-mediated inflammatory response and develop decreased insulin sensitivity.

In addition to T cells, B cells can also accumulate in adipose tissue in HFD-fed obese mice.[38] Indeed, a recent study showed that recruitment of B cells can promote the activation of T cells and potentiating the M1-like macrophage polarization. Furthermore, B cells can cause the systemic effects through the

production of pathogenic immunoglobulin G (IgG) autoantibodies.[38]

Mast cells. It was suggested in 1963 that there were an increased number of mast cells in adipose tissue of obese hyperglycemic mice.[39] This finding was recently confirmed in obese mice and humans by Liu et al.[40] Mast cells secrete a wide range of mediators such as IL-6 and IFN-γ, which could modulate the environment of the inflamed WAT.[41] Mast cell–derived IFN-γ, matrix metalloproteinase 9, and phospholipase A2[42] are among the mediators that regulate the activation of the macrophages.

Eosinophils. Adipose tissue eosinophils may have a role in sustaining the M2-like ATM polarization state, and the adipose tissue content of eosinophils is greatly decreased in obesity.[43] The resident eosinophils in the WAT were identified as the main source of IL-4 and IL-13, and in their absence, the number of M2-like ATMs is greatly reduced. Eosinophil-deficient mice show more inflammation and insulin resistance than the wild-type mice on an HFD.[30] Furthermore, hypereosinophilic mice displayed improved glucose tolerance in HFD-fed obese mice,[30] suggesting that the eosinophils help to control the adipose tissue inflammation and promote normal insulin sensitivity by promoting the M2-like ATM polarization state. Along these lines, decreased adipose tissue eosinophils in obesity could contribute to inflammation and insulin resistance.[30]

Natural killer cells. Natural killer (NK) cells are present in many metabolic tissues such as the liver and adipose tissue. The role of NK cells in obesity and WAT inflammation is not entirely clear. Although several groups reported an unchanged number of circulating NK cells in obesity,[44,45] others observed significantly lower numbers of circulating NK cells in obese subjects.[46] An intense cross talk between NK cells and other leukocytes, including the lymphocytes, macrophages, and neutrophils exists,[47] as B- and T-cell–deficient mice have a coordinated increase in NK cells and ATMs.[48] In obese people, there is evidence that the adipose tissue NK cells are a source of inflammatory cytokines such as IFN-γ or TNF-α and several chemokines including the MCP-1 and chemokine (C-X-C motif) ligand 8 (CXCL8).[49]

Neutrophils. Neutrophils are at the first defense line in immune response that infiltrate into the inflamed tissues. They promote the subsequent recruitment of inflammatory monocytes by producing MCP-1 and other chemokines. Neutrophils communicate with multiple components of both innate and adaptive immunity and are closely linked to macrophage immune function.[50] The role of neutrophils in obesity-induced inflammation is not well understood, but there are clear associations between neutrophils and obesity. Mild increases in circulating neutrophils are observed in obese adults and children.[51,52] Neutrophils are found in adipose tissue in small numbers, but migrate to fat even with short-term HFD feeding.[53]

HOW DOES OBESITY CAUSE INFLAMMATION?

Several mechanisms have been suggested to explain how obesity causes inflammation in adipose tissue. In the following section, we discuss these proposed hypotheses.

Adipokines modulate the production and release of inflammatory cytokines. Adipocytes secrete a great deal of bioactive molecules of different nature, collectively termed adipokines, many of which have immunomodulatory actions. Two main adipokines are leptin and adiponectin (Fig 3).

Leptin, the first discovered adipokine, can regulate immune function at various levels: upregulating the phagocytic function, stimulating the proliferation of human circulation monocytes in vitro and differentiation into macrophages, modulating the activation of NK lymphocytes, and inducing the secretion of proinflammatory cytokines (TNF-α IL-6, and IL-12).[54] Studies in animal models demonstrated that the absence of leptin leads to a defective immune function. For instance, leptin-deficient (ob/ob) mice have severe thymic atrophy, lower lymphocyte and NK cell numbers, decreased cytotoxic activity, and lower expression of proinflammatory cytokines. These mice and the db/db mice (mice carrying a defective leptin receptor gene) are less efficient in fighting infections.[54] Leptin is a sensor of the state of energy stores in the body, and when these stores enlarge, it acts on the central nervous system, in particular the hypothalamus, to suppress food intake and stimulate the energy expenditure.[55] Circulating leptin levels increase in parallel with an increase in body fat mass. However, it is well known that, in most cases, human obesity is accompanied by a leptin resistance and the compensatory hyperleptinemia. This leptin resistance state might have possible consequences on the activation of immune cells.[56] In this regard, adipocyte-specific downregulation of leptin receptor expression,[31] which mimics the state of leptin resistance in the adipose tissues of diet-induced obesity, causes an increase in TNF-α expression.[31,57]

Adiponectin, an adipocyte hormone with anti-inflammatory and insulin-sensitizing properties, exerts opposite immunomodulatory actions to leptin. It inhibits the phagocytic activity and the production of TNF-α in macrophages, the differentiation of monocyte precursors, the synthesis of endothelial adhesion molecules, and the formation of foam cells.[58] In addition, it

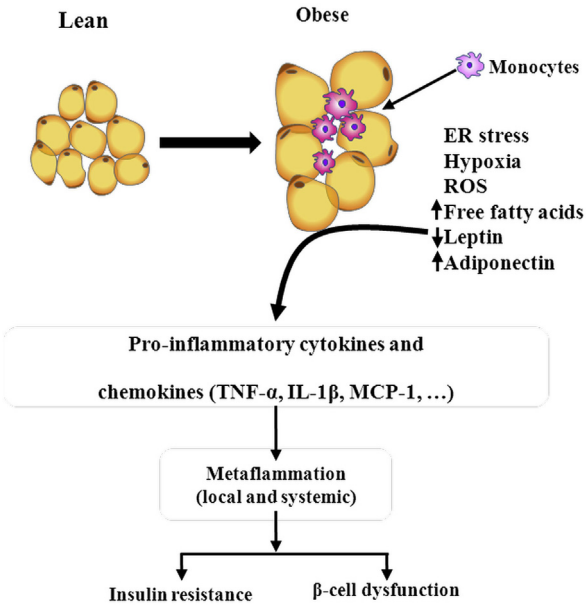

Fig 3. Relationships between adipocytes and macrophages in the context of inflammation in obesity. The hypertrophied adipocytes present altered secretion of adipokines, cytokines, and FFAs. Different factors including the changes in adipokine secretion, ER stress, increased FFA release, increased ROS production, and hypoxia have been proposed as the causes of the adipocyte inflammation. Adipocyte inflammation leads to release of proinflammatory cytokines locally and systematically that these inflammatory factors in turn could induce insulin resistance and β-cell dysfunction in T2D. ER, endoplasmic reticulum; FFA, free fatty acid; IL, interleukin; MCP, monocyte chemotactic protein; ROS, reactive oxygen species; T2D, type 2 diabetes; TNF, tumor necrosis factor.

stimulates the release of anti-inflammatory IL, such as IL-10 or IL-1RA.[59] Human studies have revealed that unlike leptin, adiponectin concentrations correlate inversely with body fat mass.[60] Taken together, the changes in the levels of these 2 main adipokines may contribute to the onset and maintenance of the systemic inflammation along with insulin resistance present in obesity.

Fatty acids can induce inflammation. Abnormally increased blood lipid levels, including nonesterified FFAs are a common feature in obesity. Adipose tissue as a whole has limited capacity to expand and store energy. Exceeding this limit leads to enhanced lipolysis within the adipocyte and the subsequent release of FFAs into the blood stream. From the blood, FFAs accumulate in tissues throughout the body, including the liver, muscle, and the pancreas, where they interfere with normal metabolism of the tissues.[61]

The chemical nature of FFAs is relevant to triggering the inflammatory response. Studies on weight-discordant twins have shown that obese individuals exhibited signs of insulin resistance and stimulated inflammatory responses in adipose tissue when compared with their lean twins. These alterations were parallel to considerable differences in adipose tissue fatty acid composition, which resulted in diminished proportions of stearic, linoleic, and α-linolenic acids, and increased levels of palmitoleic and arachidonic acids in obese twins.[62] Saturated FFAs appear to be especially important in this regard, as they may stimulate TNF-α and IL-6 production in adipocytes. Furthermore, there seems to be a positive feedback loop between saturated FFAs from adipocytes and cytokines from macrophages: saturated FFAs can increase the production of TNF-α in macrophages, and in turn the immune cells induce lipolysis in adipocytes. These events accelerate the inflammatory changes in the adipose tissue in obesity.[63]

How can FFAs elicit an inflammatory response? One of the potential mechanisms in this regard could be the effect of FFAs on adipokines production and secretion. As an example, the adiponectin levels have been negatively and positively correlated with saturated FFAs and polyunsaturated fatty acids, respectively.[64] Alternatively, FFAs may directly induce inflammatory pathways through the activation of the cell receptors. Recent evidence has suggested that saturated FFAs may act through binding to TLRs, especially TLR4. This binding leads to the activation of NF-κB pathway resulting in the induction of inflammatory responses in adipocytes.[65] In turn, the proinflammatory cytokines stimulate lipolysis and increase FFA levels, leading to the aggravation of the condition in adipose tissue.

Too many nutrients leading to cellular stress. The excessive adipose tissue growth has further consequences at the cellular and subcellular levels. Mitochondria and the endoplasmic reticulum (ER) are two adipocyte organelles that are affected in obesity. Briefly, the ER is a primary site for protein synthesis and triacylglycerol droplet formation. Under conditions of cellular stress, ER function becomes progressively impaired and this triggers a security mechanism known as the "unfolded protein response" (UPR). The state of nutrient excess and cell expansion in obesity implies a greater demand for protein and triacylglycerol droplet formation, and this may as well induce ER stress and the UPR. The disruption in ER homeostasis is sensed by three different molecular components, inositol-requiring protein 1 (IRE1), activating transcription factor 6, and double-stranded RNA–dependent protein kinase (PKR)-like ER kinase. Together, they regulate the expression of numerous genes in an attempt to alleviate the ER stress.[66] The UPR is also linked to (1) the production of reactive oxygen species (ROS), and therefore a greater oxidative stress and (2) the activation of inflammatory pathways, with increased expression of cytokines, such as IL-8, IL-6, MCP-1, and TNF-α.[65]

In hypertrophic adipose tissue, there is an increase in lipolysis, which results in an excess of cellular FFAs. This condition in combination with glucose overload results in increased activity of the oxidative pathways. Over time, this energy overload leads to mitochondrial dysfunction, which subsequently results in an increased ROS production. Such oxidative stress can then activate the immune system by inducing the redox-sensitive transcription factors such as NF-κB.[67]

The obese adipocytes need oxygen. As stated earlier, the obese adipocytes become larger and larger when they receive a greater nutrient supply. In consequence, adipose tissue expansion in obesity eventually reaches a point where the development of local vasculature is insufficient, and the tissue cannot meet the oxygen demands of distant enlarged adipocytes. Studies in animal and culture models have suggested that the hypoxic adipocytes produce inflammatory signals to stimulate angiogenesis.[68] The key element in the initiation of the cellular response to hypoxia is the hypoxia-inducible factor 1, a transcription factor highly unstable under normoxic conditions. Hypoxia-inducible factor 1 regulates the expression of a great number of genes involved in different functions including the angiogenesis, inflammation, and energy metabolism. Some of these genes are leptin, plasminogen activator inhibitor 1, and macrophage migration inhibitory factor, which all become upregulated in obesity.[68]

Hypoxia has important consequences for adipocyte metabolism, as it forces the cell to switch from aerobic to anaerobic glycolysis to obtain energy from glucose. This results in increased production and release of lactate from adipocytes. Lactate has been shown to stimulate the inflammatory pathways in macrophages[69] and enhance the LPS-induced inflammatory response in preadipocytes.[70]

Adipocytes are not the only cells responsive to hypoxia within the adipose tissue. Resident macrophages tend to accumulate around the hypoxic areas, probably recruited by chemotactic signals released from the affected adipocytes. They respond to the lack of oxygen in a similar manner to adipocytes, by producing the proinflammatory cytokines. It is apparent that the hypoxia response fails to achieve the expected effect of increasing adipose tissue vascularization, but instead it leads to a situation of local fibrosis, which contributes to adipose tissue dysfunction.[71] In line with this, hypoxia has been found to induce the UPR in cultured adipocytes.[72]

MEDIATORS OF INFLAMMATION

As stated earlier, chronic low-grade systemic and local inflammation could link the obesity to insulin resistance. In this section, we discuss the role of the mediators of the inflammatory responses in adipose tissue and its immune cell compartments.

Toll-like receptors. TLRs are a family of pattern recognition receptors (PRRs) that play a critical role in the early innate immunity by recognizing the pathogen-associated molecular patterns (PAMPs). Endogenous components derived from the dying host cells, termed damage-associated molecular patterns, can also activate the TLRs.[73] TLRs are transmembrane proteins expressed on different immune and nonimmune cells such as B cells, dendritic cells, macrophages, mast cells, NK cells, epithelial cells, fibroblasts, and endothelial cells.[74] TLRs are largely divided into 2 subgroups depending on their cellular localization. TLR1, TLR2, TLR4, TLR5, and TLR6 are localized on the cell surface and recognize microbial membrane components, whereas TLR3, TLR7, TLR8, and TLR9 are expressed within the intracellular vesicles and recognize the nucleic acids.[75] Each TLR is capable of sensing different PAMP subsets and activating distinct cellular responses.[76] TLR2 recognizes a wide array of structurally diverse PAMPs such as lipoproteins or lipopeptides.[77] TLR1 and TLR6 complex with TLR2 and discriminate between triacyl- and diacyl-lipopeptides, respectively.[78] TLR4 recognizes the major cell wall component LPS of Gram-negative bacteria. TLR activation triggers several intracellular signaling pathways leading to the activation of transcription factors, such as NF-κB and AP1, which are common to all TLRs. This activation results in production of inflammatory cytokines and chemokines.[79] Some TLRs can also activate the interferon-regulatory factors, leading to production of type 1 IFNs such as IFN-α family and IFN-β and chemokine RANTES (regulated on activation normal T cell expressed and secreted).[80]

Among different types of TLRs, TLR4 has been reported to have a key role in adipose tissue inflammation. TLR4 expression is increased in adipose tissue of obese mice and also obese and diabetic patients. Saturated FFAs and LPS from Gram-negative bacteria are potential ligands of TLR4 both in adipocytes and macrophages.[81,82] Mice deficient in TLR4 provided more evidence on the role of TLR4 in obesity-induced inflammation. A mild reduction in inflammation of different tissues especially adipose tissue has been reported from mice lacking TLR4.[83,84] This reduced inflammation in adipose tissue was due to a decrease in macrophage infiltration or a change in macrophage polarization toward an M2 anti-inflammatory phenotype.[82,84,85] Overall, these findings provide the evidence that TLR4 is a key mediator in obesity-associated inflammation.

Nuclear factor kappa B. NF-κB plays a critical role in regulating inflammation. In the basal (resting) state, NF-κB is retained in the cytoplasm by its binding to kappa light polypeptide gene enhancer in B-cell inhibitor, α (IκBα). In response to cellular stimulation, IκBα protein is phosphorylated on 2 conserved serine residues by inhibitor of kappa light polypeptide gene enhancer in B cells, kinase β (IKKβ).[86] This phosphorylation induces the proteasome-dependent degradation of IκBα leading to nuclear translocation of NF-κB. In nucleus, NF-κB transactivates the inflammatory genes by binding to specific sequences in the promoters.[86]

There are two major pathways of NF-κB activation: the canonical and nonanonical pathways.[87] The canonical pathway is initiated by activation of the PRRs, which include TLRs and NOD-like receptors (NLRs). PRRs sense PAMPs including the microbial-derived LPS, peptidoglycan, and bacterial DNA, as well as damage-associated molecular patterns such as saturated FFAs.[88]The noncanonical pathway is activated after the stimulation of TNF receptor family members such as CD40 or lymphotoxin beta receptor.[89]

Human and animal studies also support the role of NF-κB in obesity-induced inflammation. An increased activity of NF-κB in adipocytes of obese animals has been reported.[90] In addition, inhibition of NF-κB pathway by pharmacologic inhibitors of IKKβ such as aspirin or by genetic deletion of IKKβ demonstrated an improvement in obesity-induced inflammation in animals.[90,91] These results were confirmed in diabetic patients, as treatment of the patients with aspirin improved the glycemic control along with inhibiting the NF-κB activity in their PBMCs.[92] Taken together, these findings suggest a crucial role of NF-κB in obesity-induced inflammation and the inhibition of this pathway might be a potential target for treatment of insulin resistance and diabetes.

C-Jun N-terminal kinase (JNK). JNKs are members of a larger group of serine-threonine (Ser-Thr) protein kinases from the mitogen-activated protein kinase (MAPK) family involved in the cellular stress response, apoptosis, proliferation, differentiation, migration, and transformation.[93] Two major isoforms of JNK, JNK1 and JNK2, have been biochemically characterized.[94] JNKs are activated by dual phosphorylation at specific threonine (Thr) and tyrosine (Tyr) residues, in response to a variety of cellular signals, including the inflammatory cytokines, growth factors, and environmental stress. Phosphorylated JNKs activate the transcription factor c-Jun by phosphorylating several modulatory Ser/Thr sites.[95] Activated c-Jun homodimerizes and/or heterodimerizes with c-Fos generating the AP1 transcription complex, which binds to specific DNA sequences at target promoters and regulates the expression of cognate genes.[94]

JNK signaling pathway is activated by obesity.[96] It has also been suggested that JNK signaling contributes to inflammation. In this regard, Solinas et al[97] used the adoptive transfer to generate mice lacking JNK1 in either radiation-resistant (nonhematopoietic) or hematopoietically derived cells. The data of this study suggested that JNK1 deficiency protects against diet-induced insulin resistance by at least 2 mechanisms. Absence of JNK1 in the nonhematopoietic compartment prevents diet-induced obesity and leads to indirect improvement of insulin sensitivity through the maintenance of a leaner body mass. On the contrary, JNK1 deletion in the hematopoietic compartment does not affect adiposity and has a direct effect on the insulin receptor signaling but still protects against HFD-mediated insulin resistance by decreasing obesity-induced inflammation. Proinflammatory cytokines in both liver and adipose tissue of HFD-fed mice were reduced on JNK1 deletion in the hematopoietic compartment. It has also been shown that JNK1 is required for FFA-mediated induction of proinflammatory cytokine production in macrophages.[97] Gene expression analysis showed that palmitate-mediated induction of IL-6 and TNF-α messenger RNAs (mRNAs) was significantly reduced in peritoneal macrophages from JNK1$-/-$ mice.[97] In another study, Han et al[98] suggested that JNK2 in myeloid cells may play a bigger role in the development of obesity-induced insulin resistance when JNK1 is deleted. They reported that feeding an HFD to control and JNK-deficient mice caused similar obesity, but only mice with JNK-deficient macrophages remained insulin sensitive. In mice with macrophage-specific JNK-deficiency, the protection against insulin resistance was associated with reduced tissue infiltration of macrophages. Immunophenotyping demonstrated that JNK was required for proinflammatory macrophage polarization.[98] Taken together, these studies demonstrate that JNK in macrophages is required for the establishment of obesity-induced inflammation.

ER stress. As stated earlier, ER is an intracellular organelle responsible for the synthesis of polypeptides and post-translational modification and folding of peptides, synthesis of lipids and sterols, and maintenance of the intracellular calcium homeostasis. ER stress is a phenomenon that occurs when an excessive protein misfolding exists during biosynthesis. Although the UPR is an adaptive response for cells to restore the ER homeostasis, severe or prolonged ER stress has been linked to various diseases including the neurodegenerative

diseases, developmental disorders, diabetes, cystic fibrosis, infectious metabolic diseases, inflammatory diseases, cancer, cell death, and tissue damage.[99,100] ER stress has been recognized to induce inflammation and there are direct links between ER stress and both local and systemic inflammation.[101] ER stress is involved in many inflammatory disorders associated with the production of the proinflammatory cytokine IL-1β. In a study by Shenderov et al,[102] macrophages undergoing ER stress were able to drive the production and processing of pro–IL-1β in response to LPS stimulation in vitro. Inflammatory cytokines released from stressed cells may function as alarming or danger signals to communicate with other cells or to recruit the immune cells.[103] IRE1α plays a critical role in the transcription of inflammatory genes, and the interaction between IRE1α and TNF receptor–associated factor 2 (TRAF2) promotes NF-κB and JNK pathways.[104] In addition to NF-κB pathway, IRE1α/TRAF2 also can activate AP1 transcription factor, which promotes the expression of inflammatory cytokines.[105]

NOD-like receptors. NLRs comprise a large family of the intracellular PRRs. They are the key sensors of the intracellular microbes and danger signals and play an important role in infection and immunity.[106] Members of the NLR family assemble into large multiprotein complexes with two other proteins, apoptosis-associated speck-like protein containing a CARD (caspase-recruitment domain) (ASC) and procysteine-dependent aspartate-directed protease 1 (procaspase 1) to form the large multimeric protein complexes termed inflammasomes. The main function of the inflammasomes is to activate caspase 1 from procaspase 1, which leads to the processing of immature pro–IL-1 and pro–IL-18 into mature IL-1 and IL-18. Inflammasomes also regulate pyroptosis, a caspase 1–dependent form of cell death.[107] Among the family of NLRs, the NLR pyrin domain containing 3 (NLRP3) has been shown to have an important role in obesity-induced inflammation. In this regard, strong correlations between the expression of NLRP3 inflammasome–related genes and insulin resistance have been recently reported from the abdominal subcutaneous adipose tissue of obese male subjects with impaired glucose tolerance.[108] Additionally, patients with T2D have increased levels of NLRP3, ASC, IL-1β, and IL-18 mRNA and protein expression in monocyte-derived macrophages, compared with those in the healthy control subject.[109] The association between the NLRP3 inflammasome and both insulin resistance and obesity has been suggested by animal studies showing that the genetic ablation of NLRP3 improves insulin sensitivity and glucose homeostasis.[110] Ablation of the NLRP3 in mice has also been reported to protect from obesity-associated macrophage activation in adipose tissue, reduce M1-like macrophage gene expression, and increase the expression of M2-like cytokines. This effect was associated with an increase in the number of M2 macrophages in NLRP3-deficient obese mice, without affecting the M1 macrophage frequency.[111] Furthermore, the intracellular ceramide, ER stress, ROS generation, and potassium efflux have been suggested to activate the NLRP3 inflammasome, resulting in the subsequent release of IL-1β by human macrophages.[112] Collectively, these data suggest that the NLRP3 inflammasome has a substantial role in sensing obesity-associated inducers of caspase 1 activation and therefore regulates the development and the magnitude of inflammation.

INFLAMMATORY MEDIATORS LINKING OBESITY TO INSULIN RESISTANCE

Tumor necrosis factor α. TNF-α is a potent immunoregulatory cytokine produced by many cells including the adipocytes, keratinocytes, mast cells, langerhans cells, monocytes, and macrophages.[113,114] TNF-α has been implicated in the pathogenesis of a wide range of human diseases such as sepsis, diabetes, cancer, osteoporosis, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases.[114] TNF-α can activate the MAPK and NF-κB signaling pathways, which result in the release of other inflammatory cytokines such as IL-1β and IL-6.[115] Kern et al[116] showed that obese individuals have 2.5-fold more TNF-α in their adipose tissue compared with lean controls. Hotamisligil et al[117] demonstrated that TNF-α participates in obesity-related systemic insulin resistance by inhibiting the insulin receptor tyrosine kinase activity in skeletal muscle and adipose tissues. Peraldi et al[118] also reported that human TNF-α inhibits the insulin-dependent tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate 1 (IRS-1) in adipocytes and myeloid 32D cells. In addition, treatment of 3T3-L1 adipocytes with TNF-α resulted in reduced protein levels of glucose transporter 4 (GLUT4) along with a decreased activity of protein kinase B (Akt).[119] Taken together, these data demonstrated that TNF-α is a key mediator of insulin resistance in obesity, and its neutralizing might ameliorate obesity-induced insulin resistance in patients with T2D.

Interleukin 1. IL-1β is the most studied member of the IL-1 family because of its role in mediating autoinflammatory diseases.[120] IL-1 is a major mediator of inflammation that exerts its biological activities

through the IL-1 type I receptor (IL-1RI) produced by numerous innate immune cells including the macrophages and dendritic cells.[121] Concentrations of IL-1β together with IL-6 predict the risk of T2D in humans.[122] Juge-Aubry et al[123] have reported that IL-1β and IL-1RI expressions are markedly increased in WAT of obese individuals and this increased expression contributes further to weight gain because of its endocrine and paracrine effects on the hypothalamus and adipocytes, respectively. TNF-α and IL-1β synergistically enhance inflammation in WAT macrophages and adipocytes, an effect that is lost in the absence of IL-1RI.[115] It has also been reported that IL-1β reduces the IRS-1 expression through an extracellular signal-regulated kinase (ERK)-dependent mechanism at the transcriptional level and an ERK-independent mechanism at the post-transcriptional level.[124] IL-1 through its type I receptor also activates a number of transcription factors including the NF-κB, AP1, and cyclic adenosine monophosphate response element-binding protein, which are involved in inflammatory response.[125]

Interleukin 6. IL-6 is a multifunctional cytokine originally identified as a B-cell differentiation factor (BSF-2). IL-6 is produced by a variety of tissues including the adipocytes, activated leukocytes, endothelial cells, skeletal muscle, and liver. About 25% of in vivo systemic IL-6 is produced by subcutaneous adipocytes.[126] IL-6 is a classical proinflammatory cytokine that is a risk factor for the development of T2D in humans.[127] WAT expression and plasma levels of IL-6 are positively correlated with body mass index[128] and it negatively impacts the insulin signaling by promoting the serine phosphorylation of IRS-1.[129] Although an increased IL-6 secretion from WAT and the liver is unfavorable, the opposite is true for the skeletal muscle. Physical inactivity, which induces insulin resistance, is associated with a reduced skeletal muscle IL-6 expression and secretion.[130] Furthermore, the increases in plasma IL-6 level that result from the exercise are followed by increased IL-1RA and IL-10 levels.

HOW DOES CHRONIC INFLAMMATION LEAD TO INSULIN RESISTANCE?

Insulin signaling. Insulin action is initiated by an interaction of insulin with its receptor. Insulin receptor consists of 2 extracellular α subunits and 2 intracellular β subunits. Binding of insulin to α subunits activates the tyrosine kinase in β subunits. Upon the tyrosine kinase activation of the insulin receptor, autophosphorylation of the β subunit leads to the amplification of the kinase activity. It then recruits the IRS family of proteins. Phosphorylated IRS proteins serve as docking proteins for various effector molecules possessing the src homology 2 (SH2) domains.[131] Most of the metabolic and antiapoptotic effects of the insulin are mediated by the signaling pathway involving the phosphorylation of the IRSs and the activation of these SH2 domain proteins. This activation leads to the activation of multiple downstream effectors that ultimately transmit the insulin signal to a branching series of intracellular pathways that regulate cell differentiation, growth, survival, and metabolism.[132]

INSULIN RESISTANCE

Clinically, the term "insulin resistance" implies that a greater-than-normal concentration of insulin is required to maintain the normoglycemia. In literature, the term insulin resistance implies the resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity and T2D is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output.[133]

Molecular mechanisms of insulin resistance. At the molecular level, it has been known that defects in postreceptor signaling are the major cause of insulin resistance in target tissues.[134] A decreased autoactivation of the insulin receptor has been reported in muscle and adipose tissues of patients with T2D.[135] Furthermore, reduced expression of phosphoinositide (PI) 3-kinase (PI3-kinase) and protein kinase B (PKB), also known as Akt, has been described in skeletal muscle of obese and diabetic subjects.[136] Several mechanisms have been suggested to be involved in reduced expression and diminished phosphorylation of early insulin signaling molecules in the insulin target tissues of obese and diabetic patients. Serine phosphorylation of IRS proteins may lead to dissociation between the insulin receptor-IRS-1 and IRS-1-PI3-kinase, preventing the PI3-kinase activation[137] or accelerated degradation of IRS-1. This serine phosphorylation in turn decreases IRS-1 tyrosine phosphorylation.[138] A number of serine kinases such as JNK and protein kinase C (PKC) that phosphorylate serine residues on IRS-1 and weaken the insulin signal transduction have been identified. Another underlying mechanism of insulin resistance is related to induction of the inhibitory factors such as suppressors of cytokine signaling (SOCS). Finally, increased activity of phosphatases that dephosphorylate the insulin intermediate signaling molecules can inhibit the insulin signaling pathway.[139] In the following section, we will take an in-depth look at the role of aforementioned signaling pathways in linking the

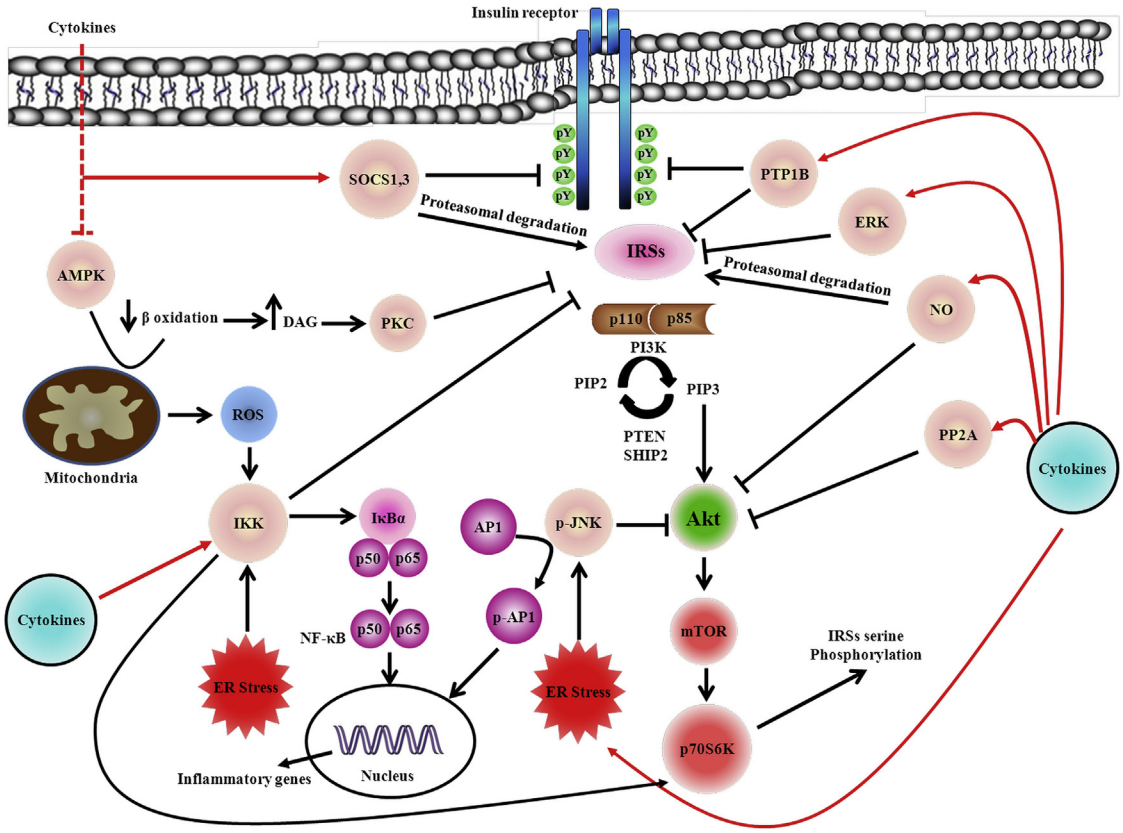

Fig 4. Molecular mechanism underlying inflammation-induced insulin resistance in insulin target tissues. Increased cytokines from the source of circulation or generated locally by tissue resident immune cells could inhibit the insulin signaling by affecting different intermediates. TNF-α can induce the activity of IKKβ, SOCS1, SOCS3, PKC, and ERK pathways leading to the serine phosphorylation of IRSs in insulin target tissues. PTP1B is another target for TNF-α and its overactivation results in dephosphorylation of the insulin receptor and IRSs. TNF-α and IL-1β have also been reported to activate the JNK, PP2A, and iNOS signaling pathways. Increased activity of these pathways leads to inactivation of Akt, an important intermediate of the insulin signaling pathway. TNF-α also activates both the IKKβ and JNK1 through a TRAF2-dependent pathway and these activations then lead to activation of NF-κB and AP1, the transcription factors that stimulate the production of many inflammatory cytokines, including TNF-α and IL-6. TNF-α also could induce the proteasomal degradation of IRSs by activating the SOCS1, SOCS3, and NO production. Finally, TNF-α induces insulin resistance by inhibiting the AMPK activity. Reduced AMPK activity suppresses fatty-acid oxidation, increases DAG accumulation, and causes insulin resistance by activating PKC. AMPK, AMP-activated protein kinase; AP1, activator protein 1; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; IKKβ, IκB kinase β; IL, interleukin; iNOS, inducible nitric oxide synthase; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa B; NO, nitric oxide; PKC, protein kinase C; PP2A, protein phosphatase 2A; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B; ROS, reactive oxygen species; SHIP2, SH2 domain-containing inositol-5-phosphatase 2; SOCS, suppressors of cytokine signaling; TNF, tumor necrosis factor; TRAF, TNF receptor–associated factor.

inflammatory factors to insulin resistance in the insulin target cells (Fig 4).

Mammalian target of rapamycin. A cellular nutrient sensor, mammalian target of rapamycin (mTOR) integrates the insulin, amino acids and nutrient signaling pathways.[140] mTOR is associated with 2 distinct protein complexes (mTORC1 and mTORC2). The mTORC2 complex is insensitive to rapamycin and includes mTOR and rictor.[141] This complex is involved in the activation of Akt and other kinases such as protein kinase A, G and C. The mTORC1 complex is sensitive to rapamycin and includes the adaptor protein raptor. The mTORC1 is the downstream of Akt and Akt-induced phosphorylation, and destabilization of the TSC1/TSC2 (tuberous sclerosis complex) is necessary for mTORC1 activation.[141] In response to insulin and amino acids, mTOR, which is a serine/threonine kinase, phosphorylates and modulates the activity of p70S6 kinase (S6K1 kinase) and an inhibitor of translational

initiation, eukaryotic translation initiation factor 4E binding protein.[142] In obesity and diabetes, mTORC1/S6K signaling is chronically activated[143] and cellular studies have shown that this chronic activation promotes insulin resistance by a negative feedback mechanism involving the serine phosphorylation and degradation of IRS-1 as well as the inhibition of IRS-1 transcription.[142,144] In contrast to wild-type littermates, transgenic mice lacking the S6K1 kinase displayed a strong resistance to age- and diet-induced obesity and insulin resistance.[145] Recently, overactivation of mTORC1 has been linked to the development of ER stress[146] a known activator of JNK pathway raising the possibility that the mTORC1/S6K and JNK pathways might cooperate in obesity and diabetes to disrupt the insulin action.

A connection between inflammation-induced insulin resistance and activation of the mTORC1 was also reported. It has been demonstrated that TNF-α impairs the insulin signaling through IRS-1 by activating the PI3-kinase-Akt-mTOR pathway.[147] Inhibition of IRS-1 tyrosine phosphorylation by TNF-α was blocked by rapamycin, an inhibitor of the mTOR. Indeed, IKKβ activated by TNF-α was shown to directly phosphorylates and inhibits the TSC1/TSC2 complex, resulting in constitutive mTOR activation.[148] This mechanism could contribute to TNF-α–induced insulin resistance through the S6K-induced IRS-1 serine phosphorylation. In hepatocytes, IL-6 activates mTOR pathway that leads to the upregulation of SOCS3,[149] a known inhibitor of the insulin signaling that decreases the insulin receptor-IRS-1 interaction and increases IRS-1 degradation. However, the functional role of mTOR in IRS-1 serine phosphorylation and insulin resistance may vary among different tissues, because a study performed by Ueno et al[150] showed that chronic hyperinsulinemia—the activator of mTOR—imposed on normal rats have a dual effect, stimulating the insulin signaling in WAT and decreasing it in the liver and muscle.

Mitochondrial dysfunction. It has been known for many years that severe mitochondrial dysfunction leads to diabetes. Mitochondrial dysfunction and consequent increases in ROS generation, in turn, activate various serine kinases that phosphorylate IRS proteins.[151] Furthermore, ROS stimulates the proinflammatory signaling by activating the IKKβ that phosphorylates IRS-1 at serine residues.[152] Although, the detailed mechanism for serine kinase activation mediated by ROS is not clearly understood, decreased ROS production by antioxidants or increased expression of uncoupling protein 2/3 improves both mitochondrial function and insulin sensitivity. In the states of obesity and T2D, mitochondrial dysfunction leads to the accumulation of FFA metabolites such as diacylglycerol (DAG) and long-chain fatty acyl-CoA.[153] Intracellular accumulation of DAG activates PKCs, including the PKCβ, δ, and θ, leading to serine phosphorylation of IRS proteins.[138,153] In fact, the PKCθ-deficient mouse is protected from fat-induced insulin resistance.[154] Regarding the role of inflammation in mitochondrial dysfunction, it has been reported that TNF-α treatment led to a decreased mitochondrial membrane potential and a reduced production of intracellular adenosine triphosphate, as well as accumulation of significant amounts of ROS.[155] In another study, among different cytokines such as TNF-α, IL-6, and IL-1β, only TNF-α treatment of 3T3-L1 cells led to an increase in oxidative stress as measured by superoxide anion production and protein carbonylation.[156] However, further studies especially in skeletal muscle and liver tissues still are required to determine whether mitochondrial dysfunction is a link between inflammation and insulin resistance in T2D.

ER stress. The experimental evidence suggests that ER stress has important role in induction of insulin resistance.[157] Two important pathways in regulation of insulin resistance, namely the NF-κB/IKKβ and JNK/AP1, are linked to activation of the IRE1 and protein kinase RNA-like endoplasmic reticulum kinase (PERK).[158] ER stress-induced activation of JNK and IKKβ kinases implicated in obesity and insulin resistance through the inhibition of the insulin receptor pathway by serine phosphorylating the IRS-1.[159] Indeed, ER stress is involved in both the dietary and genetic models of obesity and insulin resistance.[159] Activating transcription factor 6 and X-box binding protein 1 are critical regulators of ER function and its adaptive responses, as gain- and loss-of-function studies with X-box binding protein 1 demonstrated the close interaction with insulin action in vitro and in vivo.[159] Although the role of ER stress in the pathogenesis of insulin resistance is well known, less information is available about the direct effects of inflammatory cytokines on ER stress in insulin target tissues. However, authors in one study provided the evidence that TNF-α can induce ER stress in murine fibrosarcoma L929 cells. In this study, TNF-α induced the UPR in an ROS-dependent fashion.[160] Further investigations are needed to clarify the role of ER stress in inflammation-induced insulin resistance.

IκB kinase β. The IKKβ is the master regulator of NF-κB activation in response to the inflammatory stimuli. IKKβ is activated by the inducers of insulin

resistance such as the inflammatory cytokines and FFAs. It was reported that the IKKβ activity and expression are increased in obese patients.[161] 3T3-L1 adipocytes exposed to either IL-1α or IL-1β display defective insulin signaling and insulin-stimulated glucose uptake by a mechanism involving the activation of JNK1 and IKKβ via the myeloid differentiation primary response gene 88 (MyD88) signaling pathway.[124] TNF-α transiently activates both IKKβ and JNK1 through a TRAF2-dependent pathway.[162] In various insulin target cells, the inhibition of IKKβ activity prevents the deleterious effects of TNF-α or FFAs on insulin signaling.[163] At the molecular level, IKKβ promotes the IRS-1 serine phosphorylation through a direct phosphorylation[164] or through a crosstalk between the other kinases.[165] In this regard, the IKKβ activation could promote IRS-1 serine phosphorylation through the activation of TSC1-TSC2-mTORC1-S6 kinase 1 pathway.[165] Another mechanism by which IKKβ promotes insulin resistance is the induction of proinflammatory cytokine production from different cell types. IKKβ can activate NF-κB, a transcription factor that stimulates the production of many inflammatory cytokines, including TNF-α and IL-6. Thus, there is a positive feedback loop between the IKKβ and inflammatory mediators. In recent years, it was found that reduced signaling through the IKKβ pathway, either by salicylate-based inhibitors or decreased IKKβ expression, improves insulin sensitivity in vivo by affecting the local or systemic inflammation.[90] IKKβ overexpression in hepatocytes led to a local and systemic induction of proinflammatory genes and systemic insulin resistance in the absence of obesity.[166] Interestingly, neutralizing antibodies against IL-6 could reverse IKKβ-induced insulin resistance in mice.[166] Furthermore, mice lacking the IKKβ gene in hepatocytes displayed decreased liver insulin resistance along with a reduced expression of proinflammatory cytokines, but developed insulin resistance in muscle and fat in response to an HFD.[167] On the contrary, the disruption of the IKKβ in myeloid cells resulted in a systemic improvement of insulin sensitivity in a model of diet-induced insulin resistance.[167] These findings suggest that the IKKβ acts locally in liver and systemically in myeloid cells. According to these data, it appears that the major mechanism of IKKβ action in obesity-induced insulin resistance depends on the production of proinflammatory cytokines by myeloid cells, such as ATMs.

C-Jun N-terminal kinase 1. Among the different isoform of JNKs, JNK1, also called stress-activated protein kinase 1, is a key mediator of environmental stresses and inflammation. JNK activity was found to be increased in the liver, muscle, and adipose tissues of various models of obesity.[168] Activation of JNK by FFAs, stress, and inflammatory mediators such as TNF-α[169] has been shown to increase the serine phosphorylation of IRS-1.[170] An increased IRS-1 phosphorylation at Ser 307 was reported in a cellular model of insulin resistance in liver cells treated with TNF-α.[168] More importantly, no such increase could be detected in obese JNK1 knockout mice, demonstrating that the Ser 307 of IRS-1 is a target for JNK action in vivo.[168] In addition, TNF-α infusion in healthy subjects increases the phosphorylation of JNK1, concomitant with increased serine and reduced tyrosine phosphorylation of IRS-1 in skeletal muscle cells.[171] JNK1 and IKKβ are activated by the downstream of immune sensors such as TLRs and participate in the production of inflammatory cytokines via the transcription factors AP1 and NF-κB, respectively.[171] Many of the produced inflammatory cytokines are able to activate these 2 kinases leading to a feed-forward amplification loop. Several in vivo studies in mice have demonstrated the importance of the JNK pathway in the development of insulin resistance.[168,169] Whole-body JNK1 deficiency resulted in decreased adiposity, increased insulin sensitivity, and enhanced insulin receptor signaling capacity in both the genetic and diet-induced mouse models of obesity.[168] A cell-permeable JNK-inhibitory peptide improved glucose tolerance and insulin sensitivity when administered to diabetic mice and diet-induced insulin-resistant mice.[172] In addition, liver-specific overexpression of a dominant-negative JNK1 in obese diabetic mice dramatically improved insulin resistance and markedly decreased blood glucose levels; conversely, the expression of wild-type JNK1 in the liver of normal mice decreased insulin sensitivity.[173] Furthermore, blocking the JNK activation rescued the cellular and molecular defects induced by FFAs in liver resulting in the reduction of endogenous hepatic glucose production.[174] Collectively, these studies revealed JNK1 as a critical mediator linking the inflammation to insulin resistance in insulin-sensitive tissues.

Extracellular signal-regulated kinase. The ERK1/2 (also known as p44 and p42) belong to the MAPK family. The activity of ERK1/2 is increased in adipose, liver and muscle tissues of obese and diabetic patients.[175] It has been reported that the metabolic and inflammatory stresses can increase the activity of ERK in several tissues in obesity and T2D.[176] Several cellular studies have shown that the activation of ERK1/2 leads to IRS-1 serine phosphorylation.[177]

Human studies have confirmed this event, as the basal ERK activity and IRS-1 serine phosphorylation are abnormally increased in primary muscle cells from patients with T2D.[178] ERK pathway also mediates the downregulation of IRS-1 expression induced by inflammatory cytokines.[179] Activation of the ERK pathway by IL-1β in adipocytes induces a decrease in the transcription of IRS-1 mRNA, leading to a decrease in the insulin signaling activity and glucose transport.[124] ERK activation by inflammatory cytokines could also indirectly promote insulin resistance by the stimulation of adipocyte lipolysis and the release of FFAs.[180] The contribution of ERK pathway in the development of obesity and insulin resistance was first demonstrated in ERK1-deficient mice. ERK1-deficient mice are protected against diet-induced obesity and insulin resistance because of the increased energy expenditure.[164] Conversely, mice deficient in the signaling adapter p62, an ERK inhibitor, have a high basal level of ERK activity and develop mature-onset obesity and insulin resistance.[165] Another study suggested that the inhibition of ERK had beneficial effects on insulin resistance independently of an effect on body weight gain.[181]

Protein kinase C. The PKC family plays important roles in many intracellular signaling events including the cell growth and differentiation.[182] It is composed of a number of individual isoforms that belong to 3 distinct categories, conventional (PKCs α, βI, βII, and γ), novel (PKCs δ, ε, η, and θ), and atypical (PKCs ζ and λ), based on their structurally distinct N-terminal regulatory domains.[183] Recent studies have highlighted PKCs as a family of multifunctional enzymes that play crucial roles in insulin resistance in various tissues. Some are operating either as mediators (PKCζ, λ, β2, γ) or inhibitors (PKCζ, λ, α, δ, θ, ε, and β1) of the insulin signaling, depending on their activating stimuli.[182] Regarding the role of PKCs in insulin resistance, it is believed that PKC isoforms are a regulator of lipid-induced insulin resistance.

Inflammation does not seem to be a direct regulator of PKCs in the state of insulin resistance. It appears that cytokines secreted from adipocytes and immune cells indirectly affect PKCs by inducing FFA release from the adipose tissue into the circulation. Increased FFA levels in circulation then lead to lipid accumulation in peripheral tissues resulting in the activation of different PKCs. In addition, it has been reported that TNF-α increases FFA esterification into DAG ultimately leading to increased DAG content.[184] DAG is a potent activator of the PKC isoforms β and θ, which serine phosphorylate the IRS-1, resulting in insulin resistance in skeletal muscle cells. However, authors in one study suggested a direct effect of TNF-α on PKCs in adipocytes. The results of this study indicated that TNF-α inhibition of insulin-induced the insulin receptor and IRS-1 phosphorylation, IRS-1/PI3-kinase association, and glucose transport occurs, in part, via the modulation of the activity of PKC isoforms α and δ and their association with the upstream elements in the insulin signaling cascade.[185]

As stated before, lipids are the main regulators of PKCs. It has now become clear that PKCθ in muscle, and PKCε in liver relay DAG-induced insulin resistance in these tissues. Similarly, the implication of PKCζ in mediating the deleterious effects of ceramides in muscle cells and adipocytes has now been well established. Studies using lipid infusion have shown that an increase in plasma FFA levels result in an increased intracellular fatty acyl-CoA and DAG concentrations in muscle and liver, which subsequently leads to activation of the proinflammatory kinases PKCθ and PKCε, respectively.[182] Activation of PKCθ, in turn, results in increased IRS-1 serine phosphorylation, which decreases IRS-1 tyrosine phosphorylation and reduces IRS-1–associated PI3-kinase activity, leading to a reduced insulin-stimulated glucose transport activity.[138] Using the same lipid infusion technique, it was also shown that PKCθ-deficient mice were protected from fat-induced defects in the insulin signaling and glucose transport in skeletal muscle.[154]

Suppressor of cytokine signaling. The SOCS protein family also named Janus family kinase-binding proteins or signal transducer and activator of transcription-induced Stat inhibitor includes 8 members (SOCS1–7 and cytokine-induced STAT inhibitor). They play an essential role in mediating the inflammatory responses in both immune cells and metabolic organs such as the liver, adipose tissue, and skeletal muscle.[186] Various factors including the activators of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway, such as members of the IL-6 family of cytokines and IFN-γ, and non–JAK-STAT activating cytokines, such as TNF-α, and growth factors have been proposed to induce SOCS proteins in different tissues.[186] In obesity, inflammation leads to an upregulation of SOCS proteins in liver, muscle, and adipose tissues. For example, SOCS1 and SOCS3 expressions are increased in the liver, adipose tissue[187] and muscle[188] of obese rodents. It appears that the control of SOCS expression by cytokines is mediated in a tissue-specific manner, as the increased IL-6 production from skeletal muscle myotubes appears to be the dominant factor driving the SOCS3 expression in skeletal muscle of obese humans,[189] whereas in adipose tissue of rodents, TNF-α may be the most

dominant factor.[11] Several cellular studies have demonstrated that the SOCS proteins negatively regulate the insulin signaling pathway. Different mechanisms have been proposed to explain how SOCS proteins inhibit the insulin action. For example, SOCS1 inhibits the catalytic activity of the insulin receptor. SOCS3 inhibits the insulin signaling by a direct binding through its SH2 domain with the juxta membrane phosphotyrosine 960 on the insulin receptor, thus preventing the interaction of IRS-1 and IRS-2 with the receptor. SOCS proteins also target IRS proteins for proteasomal degradation. SOCS1 and SOCS3 interact with the tyrosine phosphorylated IRS-1 and IRS-2 resulting in their ubiquitination and degradation by the proteasome.[190] In vivo studies also support the role of SOCS proteins in insulin resistance. In this regard, overexpression of SOCS1 or SOCS3 in mouse liver reduced the expression of IRS-1 or IRS-2 leading to liver and systemic insulin resistance.[191] Overexpression of SOCS3 in muscle exacerbated diet-induced obesity and insulin resistance.[192] In addition, targeted invalidation of SOCS3 in adipose tissue or in muscle protected mice against obesity-induced insulin resistance.[193] SOCS7-deficient mice also displayed improved glucose tolerance and insulin sensitivity.[194] In summary, aforementioned findings suggest that the SOCS proteins are involved in the regulation of the insulin sensitivity and their targeting could be a therapeutic target for treatment of metabolic disorders.

Inducible nitric oxide synthase. Nitric oxide (NO) is an endogenous signaling molecule produced by NO synthase. NO acts as a signal transduction molecule for a number of physiological processes such as vasodilation. It is also involved in many pathophysiological states including insulin resistance. Proinflammatory cytokines (TNF-α, IL-1, and INF-γ) and endotoxins synergistically increase NO production through increased expression of inducible nitric oxide synthase (iNOS) in rat skeletal muscle, cultured myocytes, and adipocytes.[195] In the insulin signaling pathway, NO can reduce the Akt activity by causing s-nitrosylation of a specific cysteine residue.[196] Increased iNOS activity also results in degradation of IRS-1 in cultured skeletal muscle cells.[197] In addition, iNOS was increased in muscle and adipose tissues of the genetic and dietary models of obesity. In this regard, iNOS-deficient mice had improved glucose tolerance, normal insulin sensitivity, and normal insulin-stimulated glucose uptake in skeletal muscle.[198] Finally, treatment with iNOS inhibitor reversed fasting hyperglycemia and hyperinsulinemia and improved insulin sensitivity in ob/ob mice. iNOS inhibitor also increased protein expression of IRS-1 and IRS-2, and enhanced IRS-1 and IRS-2–mediated insulin signaling in the liver of ob/ob mice.[199] Thus, iNOS represents another molecular mechanism linking cytokine-mediated inflammation to insulin resistance.

Phosphatases. Another molecular mechanism underlying insulin resistance in obesity is increased expression and activity of several phosphatases in insulin-sensitive tissues. Phosphatases are enzymes that remove the phosphate entity from the cellular substrates.[200] Phosphatases are divided into different classes including histidine phosphatases, lipid phosphatases, serine/threonine phosphatases, protein tyrosine phosphatases (PTPs), and dual-specificity phosphatases that target both phospho-Tyr and phospho-Ser/Thr residues.[201] In the following section, we discuss about the phosphatases that have a role in inflammation-induced insulin resistance in insulin target tissues.

Protein tyrosine phosphatases. PTPs have been found to play an important role in the steady-state balance by catalyzing the tyrosine dephosphorylation. Although several different phosphatases have been implicated as inhibitors of the insulin action, in vivo analysis in mice strongly supports protein tyrosine phosphatase 1B (PTP1B) as the major regulator of the insulin signaling.[139] PTP1B acts as a negative regulator of the insulin signaling by dephosphorylating the insulin receptor and its substrates.[202] Obese, insulin-resistant, and diabetic patients show a high expression of PTP1B in their muscle and adipose tissues.[203] Genetic variations within the promoter and coding sequences of the PTP1B gene have been reported to be associated with insulin resistance and T2D.[204,205] In addition, transgenic overexpression of PTP1B in muscle causes insulin resistance, impairs insulin signaling, and decreases glucose uptake in this tissue.[206,207] On the contrary, PTP1B-deficient mice are resistant to weight gain, exhibit increased insulin sensitivity, and remain insulin sensitive when subjected to an HFD.[207,208] Furthermore, the inhibition of PTP1B using small interfering RNA or antisense oligonucleotide improves insulin sensitivity in db/db mice and in vitro.[209,210] Various factors such as inflammation, glucose, and palmitate have been proposed as the cause of increased expression of PTP1B in tissues of obese and diabetic patients.[211-214] Our laboratory provided the evidence that palmitate and inflammation are the main factors contributing to increased PTP1B expression in skeletal muscle cells. Palmitate and inflammation can additively induce the expression of PTP1B via a mechanism involving the activation of JNK and NF-κB pathways.[206,212,213] In this regard, brown adipocytes treated with TNF-α

showed a significant enhancement of PTP1B mRNA, protein, and activity. TNF-α has also been reported to induce PTP1B transcription via the NF-κB activation in multiple tissues in various models of obesity and diabetes.[211] Accordingly, the lack of PTP1B expression confers protection against TNF-α–induced insulin resistance in skeletal muscle either in vitro or in vivo.[215] Taken together, the aforementioned data support the idea that at least part of the effects of TNF-α on the insulin signaling may be exerted through the modulation of PTP1B expression. Our data also suggest that leukocyte antigen related (LAR), a receptor-like tyrosine phosphatase, might be another mediator of inflammation-induced insulin resistance in skeletal muscle cells. In our study, palmitate induced both insulin resistance and LAR expression, whereas LAR inhibition attenuated palmitate-induced insulin resistance in C2C12 cells.[216]

Lipid phosphatases. In the signal transduction from PI3-kinase to Akt, the 2 best known negative regulators, phosphatase and tensin homolog (PTEN) and SH2 domain-containing inositol-5-phosphatase 2 (SHIP2), interfere by dephosphorylating the phosphatidylinositol 3,4,5-trisphosphate (PIP3) at the 3′ and 5′ positions, respectively.[201] In vitro and in vivo studies have provided the evidence that SHIP2 is one of the negative regulators of the insulin signaling pathway.[217] Mice lacking the SHIP2 gene have increased sensitivity to insulin.[218] Regarding the role of SHIP2 in inflammation-induced insulin resistance less information is available. However, we recently demonstrated that SHIP2 expression can be induced by palmitate via a JNK and NF-κB–dependent mechanism.[219,220] Given the common signaling pathway through which FFAs and cytokines work, it could be suggested that SHIP2 might be a mediator of inflammation-induced insulin resistance in skeletal muscle cells.

Serine/threonine phosphatases. Protein phosphatase 2A (PP2A) is a ubiquitously expressed cytoplasmic serine-threonine phosphatase that plays an important role in regulation of a diverse set of cellular proteins, including the metabolic enzymes, hormone receptors, kinase cascades, and cell growth.[221] It has been suggested that PP2A is also involved in the metabolic actions of the insulin. Okadaic acid, an inhibitor of PP2A, can activate glucose transport and GLUT4 translocation.[222] Insulin inhibits the PP2A activity and the insulin effect on PP2A is abolished in adipocytes from diabetic rats.[223] TNF-α–induced insulin resistance is accompanied by an increase of PP2A activity in rat skeletal muscle cells.[224] In this regard, PP2A activity is increased in skeletal muscle samples from the insulin-resistant patients with T2D.[225] It appears that PP2A directly dephosphorylates and inactivates Akt and PKCλ, leading to the attenuation of glucose transport stimulation in 3T3-L1 adipocytes.[226]

Serine-threonine protein phosphatase 2C (PP2C) is also involved in inflammation-induced insulin resistance. AMP-activated protein kinase (AMPK) has an important role in regulation of skeletal muscle fatty-acid metabolism. AMPK activation results in increasing the rates of skeletal muscle fatty-acid oxidation by phosphorylating the acetyl-CoA carboxylase, leading to reduced malonyl-CoA and increased long-chain fatty acyl-CoA flux into the mitochondria.[227] A decreased rate of fatty-acid oxidation in obesity has been related to reduce skeletal muscle AMPK activity.[228] Regarding the effect of TNF-α on insulin signaling, it has been suggested that TNF-α suppresses the AMPK activity via the transcriptional upregulation of PP2C. This in turn reduces acetyl-CoA carboxylase phosphorylation, suppresses fatty-acid oxidation, increases intramuscular DAG accumulation, and causes insulin resistance in skeletal muscle.[229]

Islet inflammation. Glucose-stimulated insulin secretion is central to the physiological control of metabolic fuel homeostasis, and its impairment is the hallmark of pancreatic β-cell dysfunction. β-Cell dysfunction and death lead to hyperglycemia and consequently diabetes. Pancreatic β cells are somehow similar to immune cells, as they produce cytokines; respond to cytokines from other cells and tissues, express high levels of proinflammatory proteins such as NF-κB, iNOS, nicotinamide adenine dinucleotide phosphate oxidase, and TLR and other proteins in response to immune signals. However, unlike classical immune inflammatory cells, such as macrophages or neutrophils, β cells are very sensitive to immune attack and are highly vulnerable to oxidative stress.[230]

A huge body of evidence shows that both local and systemic inflammations have a pivotal role in pancreatic β-cell dysfunction.[231] Experimental studies of prediabetic subjects and patients with T2D show the presence of islet inflammation several years before diagnosis of the disease.[232] To explain the mechanism of β-cell failure in diabetes, several mechanisms including ER stress, oxidative stress, amyloid deposition, lipotoxicity, and glucotoxicity have been explained.[233] Interestingly, all these factors finally induce an inflammatory response, whereas some may be because of the inflammation.[234] Low-level inflammatory responses probably promote β-cell repair and regeneration. But chronic inflammatory responses with activation of autoinflammatory processes may then become deleterious.[235]

Therefore, improvement in understanding the inflammatory mechanisms leading to β-cell dysfunction and apoptosis might be helpful for the development of new therapeutic strategies to prevent or delay diabetes.[230] In this part, we intend to review the molecular mechanisms of pancreatic β-cell dysfunction with respect to inflammation in T2D.

Evidence of islet immune cells in T2D. In support of insulitis in T2D, increased numbers of immune cells and increased levels of cytokines and chemokines in islets of patients with T2D have been reported.[236,237] Interestingly, several animal models of T2D showed islet immune cell infiltration.[238] The analysis of the pancreatic sections from patients with T2D, C57BL/6 mice fed on HFD, db/db mice, and Goto-Kakizaki rats demonstrates increased numbers of macrophages within the islets.[239] Analysis of islet resident macrophages and monocyte populations shows significant increases in $CD68^+$ macrophages to be around 1.5% of islets in T2D.[238] It has also been reported that a reduction in M1-type macrophages in islets restores the expression of insulin and pancreatic and duodenal homeobox 1 (PDX1) and improves the insulin secretion index.[238] In addition, high concentrations of glucose or palmitate increase chemokine production from the islets, which promote immune cell migration and infiltration into the islets. Enhanced number of macrophages and immune cells in islet of patients with T2D explains their pathologic role and finally inappropriate function of islet β cells.[240] Possibly, early infiltration of macrophages may be helpful for islet function and plasticity.[241] However, with progression of the disease, activated macrophages lead to accelerating pancreatic islet cell dysfunction and death. The presence of macrophages may also be a consequence of β-cell death to phagocytize the dead islet tissue.[242]

As stated earlier, macrophages and immune cells exert different roles and phenotypes in different tissues and organs. Polarity analysis of macrophage activation in islets by flow cytometry shows 2 main classes of macrophages in islets: $CD11b^+Ly\text{-}6C^+$ monocytes/macrophages and $CD11b^+Ly\text{-}6C^-$ macrophages.[243] In normal conditions and in the healthy mice, $CD11b^+Ly\text{-}6C^-$ cells exhibit an M2-type phenotype and in T2D models $CD11b^+Ly\text{-}6C^+$ monocytes/macrophages are M1-type phenotype. Thus, the macrophage polarity appears to be shifted toward M1 in T2D islets.[240] Therefore, the presence and functional involvement of immune cells in islets suggest strong evidence of inflammation as a responsible factor for islet dysfunction.

Activation of islets' immune system in obesity and T2D. The earliest evidence for an inflammatory process in the pancreatic islets arose from the observation that hyperglycemia induces β-cell apoptosis.[244] By examining the underlying mechanism, it was shown that high glucose concentrations induce the expression of the proapoptotic receptor FAS (also known as CD95) on β cells,[245] which is further upregulated by IL-1β produced by β cells.[246] Therefore, IL-1β and FAS contribute to both glucose-induced impairment of β-cell secretory function and apoptosis. Further in vitro experiments revealed that exposing isolated human islets to high levels of glucose increased the secretion of IL-1β, which was followed by NF-κB activation, FAS upregulation, DNA fragmentation, and reduction of insulin secretion. Several factors such as nutrients over supply (glucose and FFAs), islet amyloid polypeptide (IAPP), IL-1β autostimulation, IL-1RA reduction, and islet-derived chemokines have been proposed as the triggers of IL-1β production from the islets.[246,247] In this section, we describe the causes of IL-1β production in the islets. Fig 4 illustrates the signaling pathways involved in production of IL-1β in β cells.

Nutrient excess. The underlying mechanisms of nutrient-induced activation of IL-1β are complex. Increased levels of circulating FFAs derived from overnutrition are thought to contribute to the progressive β-cell failure associated with T2D.[242] Palmitate attracts monocytes/macrophages to islets by activating the TLR4 signaling. To confirm this hypothesis, the infusion of ethyl palmitate to mice reduced the expression of pancreatic functional genes such as PDX1, insulin, and insulin 2 (Ins2). Interestingly, ethyl palmitate infusion in mice lacking TLR4 and its adaptor protein MyD88 had no effect on the expression of pancreatic functional genes.[243] Further flow cytometry analysis showed that only in the presence of TLR4 and MyD88, the $CD11b^+Ly\text{-}6C^+$ M1-type proinflammatory monocytes/macrophages are accumulated within the islets in response to palmitate, suggesting that the TLR4 signaling is involved in macrophage infiltration to islet.[243] Further experiments demonstrated that palmitate induces the secretion of the chemokine (C-C motif) ligand 2 (CCL2) and CXC chemokine ligand 1 (CXCL1) via TLR4-MyD88 signaling in mouse insulinoma 6 and isolated islet β cells. Recruited macrophages to the islets then secrete IL-1β and TNF-α in response to palmitate to influence β-cell function. In vivo studies supported these events, as the depletion of macrophages using clodronate-filled liposomes prevented the accumulation of $CD11b^+Ly\text{-}6C^+$ cells within the islets in response to palmitate and inhibited the downregulation of PDX1, insulin, and Ins2 and upregulation of IL-1β and TNF-α in islets.[243]

Glucose. Pancreatic β cells are very vulnerable to damage caused by hyperglycemia. Chronic exposure to abnormally high blood glucose has detrimental effects on insulin synthesis, secretion and cell survival. Several mechanisms including reduced insulin gene expression, increased ER stress, enhanced chronic oxidative stress, mitochondrial dysfunction, and increased inflammatory responses can explain the detrimental effect of chronic high glucose concentration on β-cell function.[247] Ample evidence now shows that chronic hyperglycemia induces nonimmune-mediated inflammatory pathways (eg, IL-1β, NF-κB, and FAS receptor) in islets.[248] Glucose stimulates IL-1β production through the activation of NLRP3 pathway (Fig 5). Hyperglycemia triggers the dissociation of thioredoxin-interacting protein from thioredoxin under the influence of ROS, allowing binding of thioredoxin-interacting protein to the NLRP3 inflammasome. This leads to the activation of caspase 1 and the subsequent processing of pro–IL-1β and release of mature IL-1β.[249]

Islet amyloid polypeptide. In T2D, an accumulation of amyloid occurs in the islets. There is ongoing debate as to whether IAPP is responsible for the decline in functional β-cell mass or is simply a marker of β-cell demise.[250] IAPP also known as amylin, a specific polypeptide found in pancreatic islets, consists of 37 amino acids and co-secreted with insulin by the β cells. Previous studies have shown that mice overexpressing human IAPP activate the JNK pathway resulting in the apoptosis of β cells.[251] It was also reported that IAPP aggregates may actively contribute to islet dysfunction in T2D by induction of an inflammatory response.[250] Aggregates of IAPP activate inflammasome in bone marrow–derived dendritic cells, resulting in IL-1β production in a MyD88-dependent manner.[252] Furthermore, β cells directly respond to IAPP aggregates by producing the chemokine CCL2, which then attracts macrophage to the islets.[250] Islets expressing human IAPP transplanted into diabetic mice rapidly develop amyloid deposition in islets, along with macrophage accumulation. Moreover, an IL-1β antagonist can reduce amyloid deposition, macrophage recruitment, and ultimately hyperglycemia in mice.[253]

IL-1β autostimulation. Initial IL-1β induction may be amplified by a cycle of autoinflammation. Indeed, human islets, particularly purified human β cells, are very sensitive to IL-1β autostimulation.[242] This is probably a consequence of the abundant expression of IL-1R1 on these cells. Analysis of IL-1R1 expression in numerous tissues showed that the highest levels were expressed in mouse islets and by the insulin-producing cell line MIN6 compared with the other mouse tissues.[254] IL-1β autostimulation of islets can be prevented by reducing the NF-κB activity or by blocking the IL-1R1 signaling.[242,254]

IL-1 receptor antagonist. IL-1RA is highly expressed in the endocrine pancreas of nondiabetic individuals, but it is decreased in the islets of patients with T2D, and this enhances the susceptibility of β cells to IL-1β.[233] The precise mechanisms responsible for this decrease remain to be elucidated, but the adipose tissue–derived hormone leptin might be involved, as it decreases the IL-1RA expression in human islets in vitro.[233]

Islet-derived chemokines. Islet cells produce a wide range of chemokines in the context of T2D. In vitro treatment of islets with high concentrations of glucose and palmitate increases the production of several biologically active chemotactic factors such as CXCL8 and CCL3 in human islets and CXCL1 in mouse islets.[239,254] Islets isolated from rodent models of T2D (Goto-Kakizaki rats, HFD mice, and Zucker rats) also showed an increased production of various chemokines including the CXCL1, CCL2, and CCL3.[255] Importantly, the relevance of these findings for humans is supported by evidence for the upregulation of various chemokines in laser captured nearly pure β cells from patients with T2D.[256] Although most chemokines are produced by β cells in the islets, some (eg, CXCL8) may also be produced by pancreatic α cells.[239] The precise functions of the various chemokines remain to be clarified; however, they have a crucial role in tissue infiltration by immune cells in T2D.

Molecular mechanisms by which IL-1β induces pancreatic β-cell dysfunction. At the molecular level, IL-1β–induced pancreatic β-cell dysfunction is mediated via different intermediates. Chronic treatment of pancreatic β cells with IL-1β stimulates the expression of iNOS leading to production of a high amount of NO, which affects electron transfer, reduces ATP synthesis in mitochondria, and enhances the expression of proinflammatory genes.[257] Low level of cellular ATP content finally causes a reduction in insulin secretion. It has been shown that NF-κB predominantly mediates the IL-1β- or other cytokine-induced activation of iNOS in pancreatic β cells.[258] Long time activation of NF-κB induces a sustained decrease in the expression of β-cell–specific proteins including the insulin, GLUT2, and PDX-1 concomitant with an increase in iNOS expression.[259] Overexpression of manganese superoxide dismutase with reduction in NF-κB activation and iNOS expression largely showed a protective effect on IL-1β–induced apoptosis in β cells.[260] In vivo study on NF-κB (p50)-deficient mice also showed a resistance to multiple low-dose streptozotocin-induced diabetes.[261]

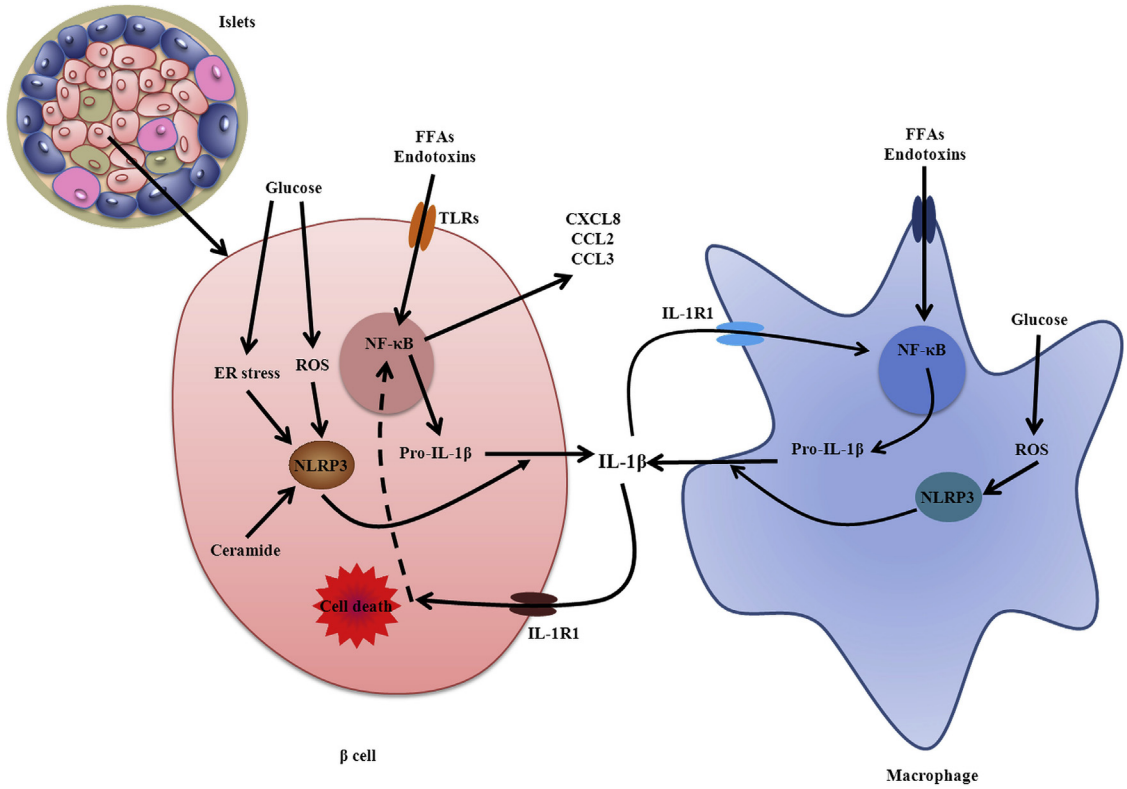

Fig 5. Molecular mechanisms underlying the islet inflammation. Increased levels of circulating FFAs such as palmitate attract monocytes/macrophages to islets by activating the TLR4 signaling. Recruited macrophages to the islets then secrete IL-1β and TNF-α in response to palmitate to influence β-cell function. In addition, FFAs could induce pro–IL-1β expression in β cells via an NF-κB-dependent mechanism. Chronic hyperglycemia also induces nonimmune-mediated inflammatory pathways (eg, IL-1β, NF-κB, and FAS receptor) in islets. Glucose stimulates IL-1β production through the involvement of NLRP3 pathway. Hyperglycemia activates the NLRP3 inflammasome via ROS and ER stress mechanisms leading to the activation of caspase 1 and the subsequent processing of pro–IL-1β and release of mature IL-1β. Secreted IL-1βs then produce a cycle of inflammation in macrophage and β cells. These events ultimately can induce pancreatic β-cell dysfunction via different mechanisms including the ER stress and NO production. ER, endoplasmic reticulum; FFA, free fatty acid; IL, interleukin; IL-1RI, IL-1 type I receptor; NF-κB, nuclear factor kappa B; NLR, NOD-like receptor; NLRP3, NLR pyrin domain containing 3; ROS, reactive oxygen species; TLR, toll-like receptor; TNF, tumor necrosis factor.

ER stress is another mechanism for IL-1β–mediated pancreatic β-cell apoptosis and death. To confirm this hypothesis, pretreatment of β cells with 4-phenyl butyric acid to alleviate ER stress significantly reduces IL-1β-induced cell apoptosis.[262] IL-1β and IFN-γ in combination markedly decrease the sarcoendoplasmic reticulum pump Ca^{2+} ATPase 2b protein expression and deplete ER Ca^{2+} stores by stimulating NO synthesis, which subsequently activates the ER stress pathway.[263] IL-1β plus IFN-γ also increase the expression of the death protein 5, which induces ER stress and consequently triggers β cell apoptosis.[264]

CROSS TALK BETWEEN THE β CELL AND THE INSULIN-SENSITIVE TISSUES

The last section of this review discusses the cross talk between the β cell and the insulin-sensitive tissues in the pathogenesis of T2D. The relative importance of insulin resistance and β-cell dysfunction in the pathogenesis of T2D was debated for a long time. Many groups have suggested that insulin resistance is the primary abnormality,[265,266] whereas others have consid-ered that reduced β-cell function is a prerequisite in the pathogenesis of T2D.[267,268] Mainly on the basis of Caucasian subjects, it has been proposed that T2D is triggered by insulin resistance, which is compensated initially by increased β-cell response. This condition eventually leads to T2D because of exhaustion of pancreatic β cells.[269,270] On the other hand, it has been reported that a diminished β-cell function is already present in different groups with increased risk of diabetes including first-degree relatives of patients with diabetes, women with gestational diabetes or polycystic ovary syndrome, and older individuals.[271,272] In

addition, T2D in East Asians is characterized primarily by β-cell dysfunction.[273,274] Insulin resistance is generally higher in Caucasians, whereas β-cell response is lower in East Asians.[275] Therefore, these studies indicate profound differences in T2D pathophysiology among different populations.

The relationship between insulin resistance and β-cell dysfunction is dynamic and largely dependent on the metabolic state that is primarily determined by glycemic and insulinemic status. Under physiological conditions, the glucose metabolism is determined by a feedback loop involving the islet β cell and insulin-sensitive tissues.[276] Insulin released in response to β-cell stimulation mediates the uptake of glucose, amino acids, and FFAs by insulin-sensitive tissues. In turn, these tissues feedback information to the islet regarding their need for insulin.[276] As the insulin resistance progresses and glucose uptake becomes impaired, the rise in plasma glucose concentration becomes excessive, but the increase in β-cell insulin secretion is sufficient to maintain the fasting plasma glucose concentration within the normal range.[277] The degree to which individuals are able to increase insulin secretion determines whether they develop diabetes.[278] This, in turn, is influenced by their genetic background, which explains why some obese and insulin-resistant subjects do not develop T2D.[279] Eventually, however, the insulin resistance becomes so severe that the compensatory hyperinsulinemia is no longer sufficient to maintain the fasting glucose concentration within the normal range.[277] The development of hyperglycemia further stimulates β-cell secretion of insulin, and the resultant hyperinsulinemia causes a downregulation of the insulin receptor leading to exacerbating the insulin resistance in insulin-sensitive tissues.[277] With persistent hyperglycemia, increased saturated FFA induces glucolipotoxic state and inflammatory responses that are detrimental to β cells. These conditions reduce the insulin synthesis and secretion, thereby compromising both β-cell structure and function. Taken together, the evolution of T2D requires the presence of defects in both insulin secretion and insulin action, and both these defects can have a genetic and an acquired component.[280] It is now clear that in any given diabetic patient, whatever defect (insulin resistance or impaired insulin secretion) initiates the disturbance in glucose metabolism, it will eventually be followed by the emergence of its counterpart.

CONCLUSIONS

On the basis of the evidence summarized in this review, we highlight a process of gradual progression to insulin resistance and β-cell dysfunction in obese states. In obesity, the expansion of the adipose tissue mass leads to an increased systemic FFAs flux, microhypoxia, ER stress, and ultimately adipose tissue inflammation. In adipose tissue inflammation, the activation of macrophages and other immune cells leads to the release of a variety of chemokines (which recruit additional macrophages) and proinflammatory cytokines. In turn, these cytokines initiate a process that causes the activation of proinflammatory pathways within the insulin target cells, such as adipocytes and hepatocytes, leading to insulin resistance in these cells. At the molecular level, several mechanisms have been suggested to be involved in inflammation-induced insulin resistance. These mechanisms include increase in the activation of JNK, IKKβ, PTP1B, SOCS, iNOS, PP2A, PP2C, and oxidative stress pathways. In addition to inflammation-induced insulin resistance within the adipose, liver, and skeletal muscle tissues, the same process most likely occurs in the pancreas. The pancreas also contains a pool of tissue-resident macrophages. These immune cells in the context of high glucose and FFAs produce inflammatory responses that ultimately induce apoptosis in the pancreatic β cells. Therefore, it could be reasonable that the strategies targeting inflammation and more specifically the tissue-specific macrophages, their recruitment, and activation could be promising options for treatment and prevention of obesity-related metabolic diseases. However, it should be noted that the field of obesity-induced inflammation is relatively new and we require more data from long-term follow-up studies in obese and subjects with T2D to illustrate the benefits of anti-inflammatory agents in treatment of T2D.

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