

MECHANISMS IN ENDOCRINOLOGY

Metabolic and inflammatory pathways on the pathogenesis of type 2 diabetes

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Abstract

Obesity is the main risk factor for type 2 diabetes (T2D). Studies performed over the last 20 years have identified inflammation as the most important link between these two diseases. During the development of obesity, there is activation of subclinical inflammatory activity in tissues involved in metabolism and energy homeostasis. Intracellular serine/threonine kinases activated in response to inflammatory factors can catalyse the inhibitory phosphorylation of key proteins of the insulin-signalling pathway, leading to insulin resistance. Moreover, during the progression of obesity and insulin resistance, the pancreatic islets are also affected by inflammation, contributing to β -cell failure and leading to the onset of T2D. In this review, we will present the main mechanisms involved in the activation of obesity-associated metabolic inflammation and discuss potential therapeutic opportunities that can be developed to treat obesity-associated metabolic diseases.

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Introduction

Type 2 diabetes (T2D) results from the combination of insulin resistance and a relative deficiency of insulin production (1). Despite the fact that both insulin resistance and insulin insufficiency may be induced by a number of factors that comprise genetic defects, sedentary lifestyle, dietary factors and endocrine disruptors, among others (1, 2, 3, 4, 5), inflammation has emerged as a unifying mechanism capable of affecting both the action and production of insulin (3, 4, 6, 7). Here, we will review the main aspects linking inflammation with T2D and point some of the therapeutic opportunities that may

emerge from the detailed characterisation of this phenomenon. For didactic reasons, we will organise the mechanisms involved in metabolic inflammation into three levels: i) triggers, ii) mediators and iii) amplifiers. Initially, we will introduce these concepts in the context of the general metabolic inflammation that accompanies obesity. Thereafter, we will present data showing that inflammation is also an important mechanism contributing to β -cell failure in T2D. Finally, we will present and discuss studies that have evaluated methods to dampen metabolic inflammation as an approach to treat T2D.

Invited Author's profile

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Historical aspects

The association between infections and the worsening of metabolic control in patients, with diabetes provided the earliest evidence to support a role for inflammation in glucose intolerance. The first detailed clinical description of diabetes complications in a patient with an infection was published in 1924 (8) and was followed by several other reports showing that different types of infection could also affect the control of blood glucose levels and whole body metabolism (9, 10, 11, 12). However, it was only in 1940 that a hypothesis was formulated placing the consumption of dietary fats in parallel with infections as causes for the defective action of insulin and glucose intolerance in diabetes (13, 14).

The search for the mechanisms linking inflammation and the consumption of dietary fats with insulin resistance was proven challenging, and in 1993, tumor necrosis factor alpha (TNF α) was first shown to be produced in the adipose tissue of obese rodents, mediating at least part of the effects of dietary fats to induce insulin resistance (15). Hotamisligil *et al.* (15) work had a revolutionary impact on the field, which resulted in the identification of a number of inflammatory factors that were either produced in the adipose tissue or were directly stimulated by dietary fats or lipopolysaccharide (LPS), playing important roles in the induction of insulin resistance (3, 16). In addition, more recently, studies have shown that inflammation plays an important role in the progressive deterioration of β -cell function in T2D (17, 18, 19).

The triggers of metabolic inflammation

The consumption of dietary fats is amongst the most important environmental factors leading to obesity and insulin resistance (20, 21). As obesity evolves, most of the long-chain fatty acids in the body are stored within the cells of the adipose tissue as esterified lipids (22). However, it is not only intracellular lipids that are increased in obesity. In fact, both the consumption of dietary fats and obesity result in increased blood levels of free fatty acids (FFAs) (22), which is a well known risk factor involved in the development of metabolic diseases (23, 24, 25).

For years, researchers have investigated the mechanisms connecting FFAs and insulin resistance, and it was only during the late 1990s that the first mechanisms began to be defined (26). It is currently known that FFAs can trigger metabolic inflammation and insulin resistance through at least four distinct mechanisms: i) activation of

endoplasmic reticulum (ER) stress (27), ii) activation of Toll-like receptor 4 (TLR4) signalling (3), iii) activation of protein kinase C ϵ (PKC ϵ)/PKC θ , PKC δ (28), and activation of Protein kinase R (PKR) (29). Most of the studies focus on adipose tissue and liver, but there is evidence of similar phenomena occurring in muscle and hypothalamus, as well.

ER stress

The ER is the organelle responsible for translating and folding up to 30% of the proteins in a cell (30). Most membrane-bound and secretory proteins are handled in the ER (30) in a process with a considerable degree of functional complexity. Depending on the cell type, a number of proteins can be synthesised in huge amounts; some proteins require covalent disulfide bonding and some are inserted into the membranes. Under certain stressful conditions, the pace of protein synthesis and folding can slow down, potentially threatening cellular viability (31). To minimise the chance of protein jamming in the ER, a sensor system has evolved – the unfolded protein response (UPR), which is activated in response to the accumulation of misfolded proteins in the ER lumen (32). In the ER membrane, there are three UPR sensor proteins, PERK, IRE1 and ATF6, which can be engaged by two distinct mechanisms: i) the consumption of chaperones, which leads to the removal of the inhibitory chaperone BiP from the ER luminal portion of the sensor proteins, promoting their activation and ii) the binding of misfolded proteins to the luminal part of sensor proteins, promoting their dimerisation and activation (30). The main consequence of the UPR is the reduction of general protein translation accompanied by the increase of ER chaperone expression. However, the prolonged incapacity to re-establish ER homeostasis results in the UPR-dependent activation of inflammation and eventually, apoptosis (27, 33).

In 2004, Ozcan *et al.* (34) showed that obesity was accompanied by ER stress (ERS) in the adipose tissue, leading to the activation of Jun N-terminal kinase (JNK). In cultured adipocytes, a similar phenomenon was obtained through the exposure to a high concentration of lipids. The activation of JNK was capable of inducing insulin resistance through the inhibitory serine phosphorylation of insulin substrate receptor 1 (IRS1). In another study, it was shown that chemical chaperones could stabilise the ER and rescue the metabolic phenotype of obese mice (35). Moreover, in addition to the activation of inflammatory signalling through JNK, ERS was shown to activate

inhibitor of kappa B kinase (IKK) (36), which demonstrates the wide range pro-inflammatory impact of this pathway. These findings not only confirmed the important role played by ERS as a trigger of metabolic inflammation, but they also provided proof of the concept that targeting ERS could offer an attractive approach to treat metabolic diseases. In general, the involvement of ERS in metabolic inflammation and insulin resistance emerges as a general mechanism occurring in most, if not all, tissues of the body, such as the adipose tissue, liver, muscle and hypothalamus (35, 36, 37, 38).

Toll-like receptor 4

TLR4 belongs to the TLR family of innate immune system receptors that respond to pathogen-associated molecular patterns (PAMPs) (39, 40). Lipid-A, a component of the Gram-negative bacterial endotoxin, LPS, is the main ligand of TLR4, inducing the activation of signal transduction through MyD88/IL1R-associated kinase (IRAK)-4 (41). However, studies have shown that saturated fatty acids, some of which are commonly found in the human diet, can also activate TLR4 inflammatory signal transduction (42, 43), resulting in the inhibition of the insulin signalling system (44).

Both, genetic and diet-induced rodent models of obesity exhibit increased expression of TLR4 in the adipose tissue (45). In lean mice, the intravenous infusion of lipids leads to the activation of NF κ B and increased expression of interleukin 6 (IL6) in the adipose tissue of WT mice but not in mice knockout for TLR4 (45). In addition, studies have shown that genetic targeting of TLR4 results in protection against diet-induced insulin resistance (45, 46), and depending on the genetic background of the mice, it can also protect against diet-induced obesity (38, 47). Most of the metabolic inflammatory phenotypes triggered by the activation of TLR4 depend on the expression of this receptor in bone marrow derived cells (48, 49). Studies have shown that the presence and the inflammatory phenotype of macrophages in tissues affected by metabolic inflammation is an important requirement for the induction of insulin resistance. These facts reinforce the pivotal role of TLR4-expressing macrophages infiltrating metabolically relevant tissues as the central cellular players in metabolic inflammation (50, 51).

Several attempts have been made to elucidate the mechanisms involved in fatty acid-induced activation of TLR4 (42, 52, 53). The greatest advance was made in 2012 when Pal *et al.* (54) reported that fetuin-A, an

inflammatory glycoprotein produced by the liver and adipose tissue (55), could act as a bridge linking FFA with TLR4, leading to its activation. Clinical studies have provided further evidence for the role of fetuin-A as a mediator of FFA-induced metabolic inflammation (56, 57, 58).

In addition to the direct effect of FFAs as triggers of metabolic inflammation through the activation of TLR4, studies have shown that blood levels of LPS are increased in experimental models of diet-induced obesity and also in lean rodents or humans fed a high-fat diet (59, 60). Although the blood level of LPS detected in obesity or after the consumption of dietary fat is not as high as in sepsis, for example, it is sufficient to activate TLR4 in macrophages and in cells of metabolically relevant tissues, such as adipocytes, hepatocytes and muscle cells (59, 61, 62). Therefore, it is currently believed that obesity and dietary fats can promote the activation of TLR4 through the direct action of FFAs in addition to the classical action of LPS (3, 59, 63).

An important piece of information regarding the activation of TLR4 in obesity and metabolic diseases was added by studies showing changes in gut microbiota of obese rodents and humans (64). According to these studies, the composition of the diet has a profound influence on the landscape of bacterial species present in the gut (65). A diet composed of large amounts of fibre-rich foods results in an increased proportion of *Prevotella*, whereas *Bacterioides* correlates positively with the consumption of a protein-rich diet (66). However, the greatest advance was provided by the demonstration that obese humans and experimental models of obesity present a shift in the relative amounts of the two most common phyla of gut bacteria, *Bacteroidetes* and *Firmicutes* (67, 68). In lean subjects, there is proportionally more *Bacteroidetes*, whereas in obese subjects there is proportionally more *Firmicutes* (68). Changes in the gut microbiota landscape result in modifications to gut physiology, which can be affected by a number of distinct mechanisms. At least two mechanisms have a direct impact on obesity: i) certain bacteria present in larger amounts in the gut of obese subjects express enzymes that improve energy harvesting from nutrients (69) and ii) obese gut microbiota can change the permeability of the intestine to nutrients and toxins (70). As a consequence of these modifications, there is increased whole body energy availability, increased absorption of fatty acids and increased gut transposition of LPS, which act in concert to accelerate body mass gain and trigger insulin resistance through TLR4.

PKC isoforms

At least three isoforms of PKC, PKC ϵ , PKC δ and PKC θ , have been implicated as links between lipid overload insulin resistance in liver and muscle respectively (28, 71). The activation of the novel PKC isoforms depends on the increase of diacylglycerol in the intracellular compartment, which is induced by increased lipid uptake (72). Upon activation, PKC ϵ /PKC δ /PKC θ can catalyse the serine phosphorylation of IRS1 in muscle (PKC δ and PKC θ) and liver (PKC ϵ), leading to the insulin resistance phenotype (26, 28, 73). Different genetic approaches have been employed with success to reduce the action of PKCs, resulting in increased insulin sensitivity and improved glucose tolerance (28, 74).

Protein kinase R

PKR was first identified as a cytosolic sensor of viral double-stranded RNA, leading to the activation of an inflammatory response aimed at eliminating the invader (75, 76). Later on, it was shown that dietary lipids could activate PKR, triggering an intracellular inflammatory response that integrates nutrient overload with metabolic pathways (29). In animal models of obesity and in lean mice submitted to a lipid overload, PKR is activated, leading to the inhibition of insulin signalling by two distinct mechanisms: i) direct phosphorylation of IRS-1 in serine residues and ii) integration with ERS, leading to the activation of JNK and thus, indirectly targeting proteins of the insulin signalling cascade (29). In addition, it was shown that PKR is also activated in metabolically relevant tissues in obese subjects, and body mass reduction resulting from bariatric surgery attenuates PKR activity (77). A study has reported that the inhibition of PKR using chemical inhibitors resulted in considerable improvement of the metabolic phenotype, which places PKR in an attractive position as a potential target for the treatment of T2D (78, 79).

The intracellular mediators of metabolic inflammation

In cells of the immune system and metabolically relevant tissues, the cellular signals generated in response to the triggers of metabolic inflammation can activate signal transducers that act as mediators of metabolic inflammation. At least three mediators have been characterised in depth: i) JNK, ii) IKK/factor kappa B (NF κ B) and iii) mammalian target of rapamycin (mTOR)/S6K. It is

important to mention that PKC ϵ /PKC θ , PKC δ and PKR can act both as triggers and as mediators of metabolic inflammation.

Jun N-terminal kinase

JNK, also known as the stress-activated protein kinase, belongs to the mitogen-activated protein kinase (MAPK) superfamily and exists as ten different isoforms (80). Stress signals, such as hyperlipidemia, cytokines (IL6, TNF α), hypoxia, toxins, heat shock and drugs, can activate JNK, which binds and phosphorylates c-Jun in two different serine residues, Ser63 and Ser73 (81, 82). Phosphorylated c-Jun forms dimers with members of Fos (c-Fos), Jun (JunB, JunD) or ATF families, resulting in the assemblage of the transcription factor activator protein 1 (AP-1), which is an important regulator of inflammation, cytokine production, apoptosis, neurodegeneration, cellular differentiation, migration and proliferation (83). Studies have shown that the isoform JNK1 is implicated in the pathogenesis of insulin resistance, T2D and obesity (80, 84). In experimental obesity, JNK1 is activated in the adipose tissue, leading to insulin resistance, because it catalyses the phosphorylation of IRS1 in serine residues (84).

In obesity, excessive dietary fats can be sensed by TLR4 in the hypothalamus, triggering activation of JNK and IKK/NF κ B in a MyD88-dependent manner or indirectly via the activation of ERS (34, 47, 85). Indeed, the UPR sensor protein IRE1 activates JNK, leading to phosphorylation of IRS1 in serine residues and contributing to the development of insulin resistance (34, 86). Moreover, JNK1 ablation, specifically in the CNS, normalises adiposity, glucose intolerance, insulin resistance and obesity in DIO mice (87). Tsaousidou *et al.* (88) developed an interesting model with constitutive activation of JNK1 in AgRP neurons, which resulted in leptin resistance and obesity when fed a high fat diet (HFD).

Several approaches have been used to inhibit JNK in T2D and obesity, and in all cases, there was at least some beneficial impact on metabolic abnormalities (revised in (3, 16)). Therefore, JNK is regarded as one of the most important intracellular mediators of metabolic inflammation.

Inhibitor of kappa B kinase/NF κ B

At least three triggers of metabolic inflammation can lead to the activation of IKK/NF κ B pathway – i) ERS, ii) TLR4 and iii) PKR (3, 16, 29). The NF κ B family is composed of

highly evolutionary conserved proteins, ubiquitously expressed in all mammalian cells. It acts as a transcription factor that controls a number of cellular processes, such as, cell growth, proliferation, cellular adhesion, apoptosis, inflammation and immune response (89, 90). Primarily, NF κ B activity is negatively regulated by protein interaction with the inhibitor of kappa B (IKK), which maintains NF κ B in the cytosol, preventing its nuclear activation and transcriptional function. In response to inflammatory cytokines, bacterial products or others stress signals, cytokines or TLR receptors activate a signalling cascade that converges on the phosphorylation of the inhibitor of the IKK complex (91). The IKK complex is composed of the kinases IKK α and IKK β , and of the regulatory subunit NEMO/IKK γ . The activation of IKK complex induces serine phosphorylation of I κ B, which allows its polyubiquitination and degradation by the proteasome machinery. The degradation of I κ B exposes the nuclear localisation sequence and the DNA binding domain of the NF κ B, allowing its translocation to the nucleus and regulation of target genes (89, 90, 92). Under normal circumstances, a signal decay and termination of NF κ B activity plays a role in limiting the appearance of deleterious effects. The protein deubiquitinase A20 and *de novo* synthesis of I κ B molecules act as important negative regulators. In addition, NF κ B can regulate the transcriptional activity of A20 and I κ Bs proteins, suggesting that NF κ B controls its own signal decay and termination (91, 93).

Several studies have implicated IKK/NF κ B in the pathogenesis of metabolic disorders, such as T2D, obesity and atherosclerosis (36, 94, 95). In experimental models of T2D and in obese, insulin resistant humans, IKK is activated, leading to the inhibitory serine phosphorylation of the insulin receptor substrate 1 (95, 96, 97). Both rodents and humans with T2D treated with inhibitors of IKK present at least a partial improvement of glucose intolerance (95, 98). Moreover, under nutrient overload, fatty acids can be sensed by TLR4 receptors, triggering activation of IKK/NF κ B signalling pathway, which leads to a burst in inflammatory responses (38, 43). It has been shown that consumption of dietary fats can lead to the activation of IKK/NF κ B in the hypothalamus, which results in the anomalous regulation of food intake and energy expenditure, therefore contributing to continuous body mass gain and further enhancing whole body insulin resistance (36). These findings place IKK/NF κ B in a strategic position as potential targets for treating glucose intolerance and insulin resistance.

Mammalian target of rapamycin/S6K

mTOR is a highly evolutionally conserved protein, which exists as the catalytic subunit of two different complexes: mTORC1 and mTORC2 (99). The role of mTORC2 is still under investigation, but there are studies showing that it can be regulated by growth factors leading to the activation of PI3-kinase/Akt signalling and the control of cellular cycle, proliferation and cellular survival (100, 101, 102). Conversely, more is known about mTORC1, which can induce cellular growth by integrating and coordinating signals arising from: i) nutrients (glucose and amino acids), ii) growth factors (insulin and IGF1), iii) energy sensors (AMPK), and iv) stress (hypoxia) (102). When activated by growth factors and nutrients, mTORC1 induces anabolism with consequent lipid, protein and nucleotide synthesis, lipid accumulation and inhibition of the autophagic catabolic process (102). mTORC1 activity induces the phosphorylation of three main target substrates: the ribosomal S6 kinase 1 and 2 (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF-4E), which regulate the initiation and progression of mRNA translation, leading to the enhancement of protein synthesis (101, 102, 103).

Studies have provided evidence for a role of mTOR in metabolic inflammation. In an experimental study, IL6 was shown to activate mTOR action through STAT3, leading to insulin resistance due to the increased expression of SOCS3 (104). The inhibition of mTOR using rapamycin was sufficient to revert IL6-induced insulin resistance (104). In another study using diet-induced obese mice, the disruption of the mTORC1 complex using a genetic approach resulted in improved insulin action due to the inhibition of the obesity-associated activation of the JNK and NF κ B pathways (105). Overnutrition is also involved in the chronic activation of mTOR-signalling, leading to lipogenesis in important metabolic tissues, such as muscle, liver and WAT. mTORC1 overstimulation triggers the S6K-dependent negative feedback loop, in which S6K phosphorylates and down-regulates the main substrates of the insulin receptor, IRS1 and IRS2, in serine residues, leading to insulin resistance (100, 103, 106, 107). In addition, it was shown that S6K1 deficiency results in enhanced insulin sensitivity, which is sustained even under prolonged exposure to a high-fat diet, supporting the important role of mTORC1/S6K in the pathogenesis of T2D.

The amplifiers of metabolic inflammation

A number of cytokines and pro-inflammatory factors can be induced and secreted by immune cells

infiltrating metabolically relevant tissues, leading to the amplification of metabolic inflammation. Studies have shown that some cells of metabolically relevant tissues, such as adipocytes and hypothalamic neurons, can also produce and secrete such inflammatory amplifiers. Here, we present data regarding the roles of three important cytokines that are involved in the pathogenesis of T2D: i) $\text{TNF}\alpha$, ii) $\text{IL1}\beta$ and iii) IL6 .

Tumor necrosis factor alpha

$\text{TNF}\alpha$ was the first pro-inflammatory cytokine implicated in the pathogenesis of insulin resistance, T2D and obesity (15). $\text{TNF}\alpha$ is mainly produced by macrophages and regulates pleiotropic functions, such as innate immunity, Th1 response, inflammation, cell differentiation, proliferation, apoptosis and energy metabolism (108, 109). $\text{TNF}\alpha$ is synthesised as a 24 kDa-transmembrane precursor (m $\text{TNF}\alpha$), which undergoes cleavage by the metalloproteinase $\text{TNF}\alpha$ converting enzyme (TACE) to a bioactive 17 kDa soluble molecule (s $\text{TNF}\alpha$). In obesity and T2D, both isoforms are increased and exert their biological functions by binding to $\text{TNF}\alpha$ receptors TNFR1 (p55) and TNFR2 (p75) in cells of the adipose tissue, liver and other metabolically relevant tissues (108, 110). Receptor activation leads to the induction of pro-inflammatory IKK/NF κ B and JNK/AP-1 signalling, which busts inflammation by inducing gene transcription of cytokines, cytokines receptors, growth factors, adhesion molecules and nitric oxide, among others. Additionally, $\text{TNF}\alpha$ can trigger apoptosis through the recruitment of caspases to the death domain of the p55/TNFR1 receptor.

The pro-inflammatory cytokines $\text{TNF}\alpha$, $\text{IL1}\beta$ and IL6 are increased in murine models of obesity and T2D, and pharmacological or genetic neutralisation of $\text{TNF}\alpha$ increased insulin sensitivity reduces hepatic glucose production, while increased thermogenesis reduces inflammation and body mass gain (47, 111, 112). $\text{TNF}\alpha$ contributes to the pathogenesis of insulin resistance through the induction of serine kinases that attenuate IR and IRS1 signalling pathways in important metabolic tissues (113, 114). Taken together, these data suggest that $\text{TNF}\alpha$ or its signalling pathway could be a therapeutic target for metabolic diseases. In fact, long-term treatment with $\text{TNF}\alpha$ antagonists (infliximab or etanercept) decreases inflammation, as well as fasting glucose, while increasing adiponectin levels; however it did not affect insulin sensitivity in obese diabetic subjects (115, 116).

Interleukin1 β

The polypeptide IL1 belongs to a family of cytokines mainly secreted by macrophages, monocytes and dendritic cells responsible for mediating immunological reactions, inflammation and tissue injury (117). The family member $\text{IL1}\beta$ (17 kDa), which is secreted early in immune responses, is initially synthesised as a 31 kDa precursor, pro- $\text{IL1}\beta$, which undergoes caspase-1-mediated cleavage to become active (118). $\text{IL1}\beta$ is one of the most important pro-inflammatory cytokines, which activates JNK/AP1 and IKK/NF κ B, leading to regulation of its own gene transcription, in addition to other cytokines, such as $\text{TNF}\alpha$, therefore boosting the inflammatory response (117).

Over the last 30 years, several studies have shown the important role of $\text{IL1}\beta$ on the pathogenesis of T2D, insulin resistance and obesity (119, 120, 121). $\text{IL1}\beta$ mediates diet-induced inflammation (121, 122). Studies have shown that murine models lacking components of the inflammasomes $\text{IL1}\beta$ or IL1R1 are protected from insulin resistance and diet-induced inflammation, supporting an important role for $\text{IL1}\beta$ activity in the development of metabolic disorders (123, 124). Several studies have used different approaches to inhibit $\text{IL1}\beta$ as an attempt to modify the course of metabolic disorders. The natural endogenous inhibitor IL1Ra ($\text{IL1}\beta$ receptor antagonist), produced by healthy resting cells, binds to IL1R1 and blocks the interaction and signal transduction of $\text{IL1}\beta$ (125). The use of a recombinant IL1Ra (anakinra) or $\text{IL1}\beta$ antagonists (gevokizumab, canakizumab and LY2189102) improved insulin sensitivity, reduced inflammation markers, corrected glycated haemoglobin levels and improved β -cell function in patients with T2D (126, 127). Taken together, all of these studies show that targeting $\text{IL1}\beta$ activity has a high impact on the treatment of obesity-induced inflammation, hyperglycaemia, and insulin resistance.

Interleukin6

Almost 30 years ago, IL6 was identified as a T-cell derived factor, responsible for differentiating the activated B-lymphocytes into plasma antibody-producing cells (128). IL6 (21–26 kDa) is a pleiotropic cytokine produced by a wide spectrum of cells, including adipocytes, myocytes, islet, endothelial and immune cells, involved in the acute phase response, inflammation, immune regulation, haematopoiesis and tissue regeneration (129). IL6 binds to its cognate receptor, IL6R , present in leucocytes, hepatocytes and many other cells (130, 131).

In addition, it can also bind to the soluble receptor (sIL6R-55 kDa) present in the serum and tissue fluids forming the complex IL6/sIL6R (130, 131). Upon binding to the transmembrane IL6R, IL6 promotes the phosphorylation of the gp130 protein in tyrosine residues, triggering the activation of JAK2/STAT3 and MAPK/PI3-kinase signalling pathway and inducing the expression of acute-phase proteins, adhesion molecules, chemokines, antiapoptotic proteins and cytokines (129, 132). The gp130/JAK2/STAT3 signalling upregulates the expression of SOCS3, which acts as a negative feedback inhibitor of JAK2 activation (129, 132).

In health, the expression of IL6 is transient, contributing to host defence and tissue injury, and decreasing when tissue homeostasis is restored. However, the chronic and anomalous production of IL6 plays an important role in the pathogenesis of several inflammatory diseases, including obesity, insulin resistance and T2D (131, 133, 134). Interestingly, IL6 can act as both a pro- and an anti-inflammatory cytokine, depending on the target tissue and metabolic state. Elevated plasma levels of IL6 are correlated with T2D development, body mass gain and circulating FFA, as seen in diabetic patients (134, 135). Additionally, in adipose tissue and liver, IL6 has pro-inflammatory properties, inducing insulin resistance by enhancing SOCS3 expression, which impairs IR/IRS1 phosphorylation (113, 136, 137). Thus, IL6 positively modulates insulin resistance and inflammation when secreted acutely, during exercise, or negatively when secreted chronically, as seen in T2D and obesity.

Metabolic inflammation and pancreatic islet dysfunction in T2D

Systemic metabolic inflammation can affect pancreatic islets through distinct mechanisms, contributing to β -cell failure in T2D (4). Early studies focused on the adipose tissue as the source for inflammatory factors, leading to pancreatic islet dysfunction in obesity (138). In humans, the removal of part of the visceral adipose tissue has a profound impact on whole body glucose homeostasis, mostly because of improved first phase insulin secretion (139). However, inflammation associated with obesity can affect insulin secretion by other mechanisms. Obesity-associated hypothalamic inflammation is accompanied by the loss of the first phase of insulin secretion (140). Furthermore, intracerebroventricular administration of saturated fatty acids or TNF α can induce a dysfunctional increase in insulin secretion, which is accompanied by the increased expression of apoptotic markers, such as BAX,

and also proteins involved in mitochondria function, such as PGC1 α and UCP2 in pancreatic islets (140). The increased expression of UCP2 is responsible for affecting glucose-induced insulin secretion due to the reduction of ATP production (140). The connection between the dysfunctional hypothalamus and pancreatic islets is dependent on sympathetic innervation and sympathectomy is sufficient to restore insulin secretion (140).

It has been suggested that pancreatic islet inflammation is associated with very early insulin resistance. Using a nonhuman primate model exposed during foetal life to a fat-rich diet, there was an early increase of IL1 β and IL6 expression in pancreatic islets, which was associated with a decreased first-phase insulin secretion, increased fasting glucose levels and infiltration of the pancreatic islets with macrophages before the onset of glucose deregulation and obese phenotypes (141).

The multiprotein activation complexes – inflammasomes – regulate the processing and release of proinflammatory cytokines during insulin resistance and T2D. The recruitment of pro-caspase-1 to inflammasomes leads to caspase-1 activation, which induces the proteolytic maturation and secretion of active IL1 β (142). Inflammasomes can be activated by PAMPs and damage-associated molecular patterns, such as aggregates of insoluble islet amyloid deposits, derived from the amylin peptide (IAPP) secreted by β -cells. In humans, IAPP increases the transcription of IL1 β in macrophages and contributes to islet inflammation (143, 144). IL1 β has been described as playing a central role in inflammation in the absence of infection (sterile inflammation) and its secretion has a negative impact on β -cell function and survival (122). There is a strong evidence showing that the recruitment and activation of IL1 β -producing macrophages mediate islet inflammation (122). A study demonstrated increased numbers of immune cells in the pancreatic islet of T2D patients and in animal models of obesity (138). Additionally, β -cells producing and secreting IL1 β have been observed in pancreatic sections obtained from patients with T2D, suggesting an autocrine effect of IL1 β on β -cell survival and function (120, 145). Interestingly, leptin, which is increased in obesity, can act as a proinflammatory cytokine, inducing β -cell apoptosis and generating an imbalance between IL1 β and the endogenous antagonist of the IL1 receptor (IL-1Ra) in human islets (146).

ERS has been described as an important mechanism leading to pancreatic islet inflammation and β -cell dysfunction (4, 147). The first piece of evidence of the role of ERS in defective insulin secretion came from studies with monogenic forms of diabetes (148, 149). However, it

was subsequently shown that the increased demand for insulin production in common forms of T2D could also impose perturbations in the ER, leading to the activation of the UPR (150, 151). Furthermore, as in other tissues (147), FFAs can act as a trigger of ERS in pancreatic islets, acting in concert with high glucose (19, 152).

Oxidative stress is also an important mechanism leading to β -cells dysfunction and death in T2D (153). In β -cells, mitochondrial activity is more than two times greater than in any other cell, since insulin secretion is coupled with exogenous glucose sensing and endogenous glucose oxidation in mitochondria (120). Moreover, β -cells are particularly sensitive to oxidative stress since the expression of antioxidant enzymes is lower compared to other metabolically active tissues (154). Reactive oxygen species (ROS) generation in β -cells induces the activation of stress kinases, such as JNK1. ROS also initiates the formation of inflammasomes through its association with thioredoxin-interacting protein (TXNIP) released from the complex with thioredoxin, which activates IL1 β processing (155, 156).

Recent evidence suggests that products from arachidonic acid metabolism are also involved in the generation of ROS in β -cells. 12-HETE, a 12-lipoxygenase (12-LO) product, is involved in NADPH oxidase-1 (NOX-1) activation in mouse and human islets (157). The use of 12-LO inhibitors reduces ROS and restores glucose-stimulated insulin secretion in response to proinflammatory cytokines through the attenuation of NOX-1 expression (157). In addition, in pancreatic 12-LO-knockout mice fed a HFD, the major transcriptional activator of antioxidant enzymes superoxide dismutase and glutathione peroxidase, the nuclear factor erythroid 2-related factor 2 (Nrf2), was present in high levels, demonstrating the role of 12-LO and its products in oxidative stress in pancreatic islets (158).

As a whole, pancreatic islet inflammation can be triggered by distinct mechanisms, which occur frequently in obesity. It is expected that therapeutic approaches targeting islet inflammation may be useful for delaying or interrupting the progression towards relative insulin insufficiency, which is a hallmark of T2D.

The potential role of anti-inflammatory approaches to treat T2D

A number of experimental studies have employed distinct pharmacological and genetic methods to inhibit metabolic inflammation. In many circumstances, there were beneficial outcomes of such inhibitory approaches and

many of them have been cited in the preceding sections. However, only a few clinical studies were designed and performed to evaluate the impact of anti-inflammatory approaches in human T2D.

An observational study reported improved insulin sensitivity in patients under prolonged use of immunoneutralising antibodies against TNF α to treat psoriatic or rheumatoid arthritis (115). With this concept in mind, a randomised, placebo-controlled, six-month intervention was undertaken and etanercept was shown to improve fasting glucose and increase adiponectin levels in obese subjects (116). The inhibition of IL1 β was also evaluated in humans with metabolic disease. In a double-blind, phase II, randomised study, the immunoneutralisation of IL1 β for 12 weeks reduced fasting glucose and HbA1c levels in patients with T2D (126). In addition, inhibition of the IL1 receptor using a recombinant receptor antagonist resulted in reduced HbA1c levels and increased C-peptides, suggesting a beneficial effect for the pancreatic β -cell (159).

Attempts have been made to inhibit NF κ B for the treatment of T2D. Early observational data reported improvement of glucose tolerance during the use of salicylates (160). However, side effects were always a concern and to date, no inhibitor of NF κ B is approved for the treatment of T2D. Recently, salsalate, a non-acetylated pro-drug form of salicylic acid with fewer side effects was employed in several clinical trials with generally positive results, reducing fasting glucose and HbA1c (161, 162).

An additional issue regarding the future perspectives for employing anti-inflammatory approaches to treat patients with T2D is the pleiotropic nature of the subclinical inflammatory process in metabolic diseases. It is possible that optimal effects of anti-inflammatory approaches are to be achieved on individualized basis only (163). Therefore, further studies are required to provide advance in the pathophysiology of metabolic inflammation.

Conclusions

Currently, obesity- and diet-associated inflammation is considered an important inducer of insulin resistance and defective β -cell function. As most patients with T2D are obese, prevention must be focused on reducing the prevalence of obesity. However, pharmacological approaches aimed at restoring glucose tolerance may benefit from the development of drugs that can reduce metabolic inflammation without important side effects.

It is expected that in the near future, new drugs with anti-inflammatory activity will be evaluated in clinical trials for the treatment of T2D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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