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ΘΕΜΑ: <<Bibliographic Review on the Inflammasome's role in macrovascular complications of Diabetes Mellitus and novel therapeutic strategies>>

<< Βιβλιογραφική ανασκόπηση του ρόλου του φλεγμονοσώματος στις μακροαγγειακές επιπλοκές του Σακχαρώδη Διαβήτη και των νεότερων θεραπευτικών επιλογών>>

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Abstract

Diabetes Mellitus (DM) ranks between the most common non-infectious causes of death worldwide. Today, diabetes is acknowledged as a multifaceted and diverse condition that can impact individuals at various stages of life. The umbrella of diabetes encompasses various metabolic disorders, all sharing hyperglycemia as a common denominator. Type 2 Diabetes Mellitus (T2DM) is the predominant form of diabetes, accounting for the majority of all cases. T2DM is characterized by insulin resistance, which results in hyperglycemia and, subsequently, hyperinsulinemia as the body attempts to counteract the increase in blood glucose levels. A combination of evolving lifestyles, technological advancements, and societal progress has driven an unprecedented global surge in T2DM over the past decades, elevating it to epidemic proportions.

Diabetes-related macrovascular and microvascular complications, such as coronary heart disease, cerebrovascular disease, heart failure, peripheral vascular disease, chronic kidney disease, diabetic retinopathy, and cardiovascular autonomic neuropathy, contribute significantly to the reduced quality of life, disability, and early death associated with diabetes. Due to the significant clinical burden of diabetes as a cardiovascular risk factor, there has been increasing attention on its complications. Gaining insight into the intricate interplay between genetics, environmental factors, and phenotypic expressions that contribute to diabetes could pave the way for the development of personalized treatments.

Numerous studies have clearly shown that chronic tissue inflammation plays a crucial role in the development of T2DM. Elevated levels of inflammatory proteins have been identified as predictors of T2DM. Inflammation is the body's initial

immune response to infection or tissue damage and is a key factor in the pathogenesis of various vascular diseases, including atherosclerosis. Key research into the nucleotide-binding oligomerization domain-like receptors (NOD), leucine-rich repeats (LRR), and pyrin domain-containing protein 3 (NLRP3) inflammasome complex suggests that it serves as a trigger for initiating inflammatory responses, playing a critical role in endothelial cell dysfunction and T2DM development. NLRP3 is an intracellular sensor that detects danger signals, including ischemia and both extracellular and intracellular alarmins, in response to tissue injury. As knowledge about inflammasomes continues to grow, the potential for developing new and effective therapies for patients with metabolic diseases like T2DM increases. This could open up promising new therapeutic avenues for the prevention and treatment of T2DM.

The goal of this review is to highlight the mechanisms of NLRP3 inflammasome activation and its significance in the development of T2DM and the subsequent chronic macrovascular complications that arise. At the same time reference will be made to new promising therapeutic approaches targeting the NLRP3 for the management of T2DM.

1.Introduction

Diabetes Mellitus (DM), a chronic disorder of the endocrine system characterized by abnormally high levels of circulating plasma glucose, ranks between the most common causes of death worldwide and is estimated to affect approximately 700 million adults by 2045.(1) Both environmental and genetic factors have recognized contribution to the pathogenesis and complications of DM - the former being associated with the increasing rates of obesity in adults, physical inactivity, tobacco smoking and alcohol consumption.(2) Type 2 Diabetes Mellitus (T2DM) is the most common type of diabetes attributing for the 90% of cases. Chronic complications of DM, macrovascular and microvascular, such as coronary artery disease (CAD), heart failure (HF), stroke, peripheral artery disease (PAD), diabetic retinopathy, renal disease and neuropathy account for the impaired quality of life, disability and morbidity, especially in patients with T2DM.(3)

Epidemiologic studies have recognized sub-clinical, systemic, low -grade inflammation as a pivotal feature of DM and its complications. Although the precise mechanisms of inflammation in DM are yet poorly understood, hyperglycemia, insulin resistance and excess fatty acids seem to precipitate the production of reactive oxygen species, alter intracellular signaling and increase advanced end glycation products resulting in a prothrombotic state, vascular inflammation and atherogenesis. (1,2,4–6)

An increasing amount of research on the development of diabetic complications has focused on inflammasomes—multi-protein scaffolding complexes that play a key role in regulating the inflammatory response.(2) Inflammasomes are large

intracellular multi-protein assemblies, typically with a molecular mass of at least 700 kDa. They consist of caspase-1, apoptosis-associated speck-like protein (ASC), and a nucleotide-binding oligomerization domain-like receptor containing a pyrin domain (NLRP).(7) When activated, they trigger caspase-1 activation and the release of interleukin-1β (IL-1β) and interleukin-18 (IL-18). Additionally, they initiate gasdermin D-mediated pyroptosis, a form of inflammatory programmed cell death. IL-1β has been identified as a key player in the development of Diabetes Mellitus' complications.(8) In the past few years, the scientific community has made efforts to develop molecules that can specifically inhibit the NLRP3 inflammasome. The development of NLRP3 inflammasome inhibitors has paved the way for targeting the harmful effects of NLRP3 in the onset of T2DM.(9)

In this review, we explore the mechanisms behind inflammasome activation and regulation in T2DM, while providing an overview of current knowledge regarding its role in the development of macrovascular complications. Additionally, we examine how insights into the regulation of inflammasomes by metabolic danger signals could pave the way for innovative therapeutic approaches aimed at targeting inflammasomes for more effective management of diabetic complications.

2. Inflammasomes

2.1 General overview

Inflammasomes can be described as perplexed proteinic organelles of the cytosol that coordinate the inflammatory response provoked by both microbial and non-microbial stimuli. The term ‘inflammasome’ was, firstly, introduced in 2002 in an effort to characterize the high molecular-weight assembly that holds a significant role in the innate immune response.(10) A typical structure of the inflammasome configuration would comprise of a pattern-recognition receptor (PRR) that works as a sensor protein, the apoptosis-associated speck-like protein (ASC) that contains a caspase activation/recruitment domain (CARD) and a pyrin domain (PYD), functioning as an adaptor, and the proinflammatory caspase-1(pro caspase-1).(11) Two types of PRRs have been verified, those attached to the membrane receiving extracellular signals such as Toll-like receptors (TLRs) and C-type lectin receptors and those found in the cytosol such as NOD-like receptors (nucleotide-binding oligomerization domain-like receptors) also known as nucleotide-binding leucine-rich repeat receptors(LRR) that contain (NLR) family members (NLRP1, NLRP3, NLRP4, NLRP6, NLPR7, NLPR12), absent in melanoma 2 (AIM2)-like receptors and tripartite motif (TRIM) receptors. The NOD-like receptor is coded by twenty-two genes in human species and is the first family of sensor proteins to form inflammasomes.(10–12)

Inflammasomes are an indispensable part of the immune system. They have drawn considerable interest in the past couple of decades due to their engagement in many microbial and inflammatory diseases.(12) PRRs can recognize both microbial and non-microbial danger signals, thus, promoting the activation of the inflammasome cascade. However, their importance in host's survival extends beyond infectious background. Specifically, activating stimuli include pathogen-associated molecular patterns, known as PAMPs, as well as host generated damage-associated molecular patterns such as extracellular nucleotides (ATP), reactive oxygen species (ROS), uric acid crystals, β amyloid islets, known as DAMPs.(12–15) DAMPs are released in response to tissue stress or injury, and act as 'caution mechanisms' to enhance the inflammatory response. Stimulation of the sensor protein by any of the above-mentioned activators triggers oligomerization of the inflammasome leading to the recruitment of the ASC via interaction of their PYD domain. Subsequently, ASC further oligomerizes, creating a platform for the recruitment of pro-caspase-1 via CARD-CARD interactions to activate capsase-1 which mediates the cleavage of pro-IL-1b and pro-IL-18 cytokines to their engaged forms, IL-1 and IL-18 respectively. These cytokines coordinate the innate and acquired immune responses ultimately leading to the elimination of the pathogen via pyroptosis.(12–16).

Pyroptosis is a lytic form of programmed cell death, distinct from apoptosis and necrosis, associated with the inflammatory response, typically triggered by infection or cellular damage.(17) Its basic physiological characteristics are cell membrane pore formation, rupture of the membrane, cell swelling and release of the intracellular contents. The dominant executor of the pyroptosis' pathway is the protein gasdermin D (GSDMD). Cleavage of GSDMD by caspase-1 mediates the cellular death.(18)

2.2 The NLRP3 Inflammasome

Although the NLRP3 was not the first inflammasome to be brought to light, it is indeed the most thoroughly researched due to its fundamental role against bacteria, fungus, viruses and its correlation to the development of metabolic diseases including obesity, diabetes, hypertension, atherosclerosis, gout and stroke. It is, however, more complex than was originally thought, as the inflammasome is moreover associated with non-infectious signals, with some of them being lysosomal destabilization, ROS formation and the release of mitochondrial RNA.(12,15,19,20) While a commensurate inflammatory response acts protectively against tissue damage, immoderate NLRP3 activation results in deleterious effects(16). The NLRP3, also known in the bibliography by the name of cryopyrin, is found in the myeloid lineage (dendritic cells, monocytes, neutrophils, macrophages), T and B cells, osteoblasts, fibroblasts, melanoma cells, central nervous system and epithelial cells.(10,12,21) Three main constituents form the anatomy of the NLRP3 protein, a leucine-rich repeat (LRR) located at the C-terminal that 'captures' PAMPs and DAMPs igniting the inflammasome mechanism, a central nucleotide-binding and oligomerization domain NACHT that holds ATPase properties and a pyrin domain located at the N-terminal.(13,22,23)

The activation of the NLRP3 inflammasome is at its core the autocleavage of caspase-1. Upon activation, NLRP3 self- oligomerizes, engages the apoptosis-associated speck like protein (ASC) via their respective PYD domains leading to the polymerization of ASC into filament-like structures. Following that, pro-caspase-1 is

recruited via CARD -CARD interaction resulting in the autocatalytic activation of caspase-1. Caspase -1, then, incites the proteolytic activation of the pro-inflammatory cytokines IL-1, IL-18 and gasdermin B. The later, ultimately, triggers cell death via pyroptosis. (16,19,20,22)

According to the up-to-date literature, three distinct pathways of NLRP3 activation have been studied, canonical, non-canonical and alternative. However, there are substantial understanding gaps that need to be clarified relevant to the activation and function of the NRLP3 in various non-physiological conditions.(24)

2.2.1 Canonical activation of NLRP3

In the canonical model of NLRP3 activation a two-step process is required, priming and activation.(12) That is considered as a 'safety' mechanism in order to prevent inordinate activation of the inflammasome and its consequent inflammatory and pyroptotic properties.(16) Inactive macrophages contain the NLPR3 protein, at an inert form, at low levels. Priming, a distinctive feature among NLRP protein complexes, augments NLRP3 levels and recruits macrophages for activation.(24) This preliminary priming step can be set off by Toll-like receptors (TLRs), NRLs (such as NOD-1 & NOD-2) and cytokines (such as TNF-α). These detect PAMPs, DAMPs and environmental stress leading to amplified transcription of the NRLP3, pro-IL-1b and pro-IL-18 via NF-κB transmission.(12,23–25) Though current data suggest that priming has an additional role of regulating the NLRP3 at a post-transcriptional level.(2) This complementary modulation includes modifications such as ASC phosphorylation and NLRP3 de-ubiquitination.(21) A plethora of long non-coding

RNAs and microRNAs seem to interfere with the post-transcriptional expression of the inflammasome by either enhancing or downgrading the NLRP3 signaling.(16)

Succeeding priming, an activation signal oligomerizes the NLRP3 monomers via their NACHT domains leading to the engagement of the ASC protein and provoking a chain of events that ultimately facilitate the cleavage of IL-1 and IL-18 to their biologically active forms.(23) However, the activation process as a whole is yet poorly comprehended. To this date, no common accord has been reached on the activation of the NLRP3.(24) As stated before, an abundance of PAMPs and DAMPs are capable of triggering the NLRP3 by commencing numerous molecular and cellular events. Nonetheless, none of these interfere with the inflammasome promptly.(22) Suggested pathways across the literature include ion flux, production of reactive oxygen species (ROS), lysosomal membrane rupture, NLRP3 translocation to the mitochondria and the release of mitochondrial DAMPs.(8,13,20–22,24,25)

2.2.1.1 Ion flux

Efflux of intracellular K^+ was one of the initial events to be acknowledged as a decisive step of NLRP3 activation.(24) Most NLRP3 stimuli provoke K^+ efflux in macrophages and monocytes. More specifically, the maturation and discharge of interleukin-1 in response to the NLRP3 activation is facilitated by cytosolic K^+ depletion. (12) In defense of this model, inhibition of potassium ion (K^+) efflux by high extracellular concentration of K^+ suppresses the NLRP3 inflammasome activation subsequent to its most activators.(24) Extracellular ATP is a known NLRP3 activator.(16) The K^+ efflux occurs through a pyrogenic P2X7-ATP dependent pore that recruits a pannexin-1 hemichannel. The binding of ATP to the P2X7 opens the

channel pore causing K^+ efflux that leads to morphological changes of NLRP3, opening the way for its interaction with ASC.(16,21) Although numerous studies conclude that decreased cytoplasmic potassium concentration ignites the NLRP3 inflammasome while high extracellular potassium concentration prevents NLRP3 complex activation, the molecular pathway that interlinks these outcomes with the levels of intra- or extra- cellular K^+ requires further explanation.(21) In support of that, recent research has led to the discovery of small chemical compounds, imiquimod and CL097 both of them targeting the mitochondria, that activate NRLP3 independently of K^+ efflux suggesting that NLRP3 inflammasome activation may either result from a process following K^+ efflux or occur via alternative pathways which do not depend on K^+ efflux.(12,13)

Ca^{2+} mobilization has proven involvement in the regulation of NLRP3 inflammasome.(25) Various signals such as extracellular ATP, nigericin and particulate matter can trigger Ca^{2+} mobilization either from the endoplasmic reticulum (ER) or the extracellular space, inducing mitochondrial damage, subsequent mtROS production and activation of NLRP3.(13,16) A calcium-sensing receptor (CaSR) seems to mediate the increase in intracellular Ca^{2+}. Nonetheless, the high cytosolic Ca^{2+} concentration is not sufficient to activate NLRP3 inflammasome.(13)

Apart from K^+ efflux and Ca^{2+} mobilization, the efflux of Cl^- has also been attributed to NRLP3 activation.(24) Chloride intracellular channel proteins present in both the cell membrane and the cytosol seem to facilitate the Cl^- efflux. Increased levels of extracellular Cl^- avert, whereas diminished levels of Cl^- induce NLRP3

activation. However, numerous inconsistencies have been found in the insights on K^+, Ca^{2+} and Cl^- flux suggesting that there is still further investigation needed. (13,24)

2.2.1.2 Reactive oxygen species (ROS)

A different model indicates that the production of ROS is instrumental in the NLRP3 inflammasome activation.(21) Almost all NLRP3 activators, although different in their individual characteristics, demonstrate a common property in that they all stimulate the production of ROS, causing oxidative stress in cells.(24) ROS consist of superoxide anion radical (O_2^-), hydroxyl radical (OH^-), peroxyl radical (RO_2^-), and alkoxyl radical (RO). These radicals interfere with molecules in the cell altering their structure or function. The involvement of ROS in NRLP3 activation is highlighted by the fact that ROS inhibition by specific scavengers hinders the inflammasome activation.(21,24) Additionally, studies have brought to light that thioredoxin interacting protein (TXNIP) activates NRLP3 by binding to it in an ROS-dependent manner suggesting a molecular connection between ROS production and inflammasome activation. TXNIP is a protein found in the cytoplasm that inhibits the disposition of H_2O_2, thus promoting the buildup of oxidative stress.(11,16,20,21,24) However, current bibliography emphasizes that the causative relation between ROS and NRLP3 activation needs to be verified with methods different to ROS inhibitors as the latter frequently produce various off-target effects that might lead to the observed inhibitory effect.(24)

2.2.1.3 Lysosomal membrane rupture

A third model supports that cytoplasmatic leak of lysosomal contents activates the NLRP3 inflammasome.(16) Macrophages remove particles and crystals, either formed in tissues or coming from external sources, through the process of phagocytosis. Nevertheless, the acidic environment of lysosomes makes most particulate molecules and crystals resilient to enzymatic degradation. Incomplete phagocytosis results in aggregation of engulfed particulates in lysosomes, subsequent lysosomal swelling, leakage and eventually rupture. Cathepsin B, a lysosomal cysteine protease, mediates the release of lysosomal effluent into the cytosol amplifying the NLRP3 complex activation.(11,13,16,21,24,25) Studies have shown correlation between lysosomal damage and ion efflux. In particular, lysosomal rupture induced by Leu-Leu-OMe, a soluble lysosomotropic dipeptide, was followed by K^+ efflux and Ca^{2+} influx suggesting convergence between lysosomal leakage and ion flux in the activation of NLRP3.(24)

2.2.1.4 Mitochondrial dysfunction

The accumulation of impaired mitochondria has also been proposed as a fundamental model of NLRP3 activation.(20) Upon injury, mitochondrial DNA is released from cells into circulation acting as a DAMP for NLRP3 activation.(24) Damaged organelles inside the cell, not cleared due to deficient autophagy, lead to accumulation of dysfunctional mitochondria and mitochondrial DNA (mtDNA) release, activating the NRLP3 subsequently. Mitochondrial ROS (mtROS) could also

ignite the NLRP3 complex. More specifically, studies have shown that blockage of key enzymes of the respiratory chain led to inhibition of mitophagy, mtROS buildup and NLRP3 activation.(13,19) Besides supplying ROS and DNA as DAMPs, mitochondria can also function as a docking site for inflammasome assembly.(24) The mitochondrial lipid cardiolipin has been shown to directly interact with NLRP3 and trigger its activation, indicating that cardiolipin may act as both a mitochondrial docking site and an activating ligand for NLRP3. However, cardiolipin engages in various mitochondrial processes linked to NLRP3 inflammasome activation. Therefore, whether cardiolipin directly activates NLRP3 requires further investigation.(13)

2.2.2 Non-canonical activation of NLRP3

The term ‘non-canonical activation’ was initially used in 2001 by Kayagaki et al to describe a mechanism in which lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, and caspase-11, the murine homolog of human caspases-4 and -5, led to NRLP3 activation and subsequent pyroptosis.(10,22,25) During non-canonical NLRP3 activation, cytosolic LPS (released from the phagocytosis of bacterial walls during infection) directly interacts with the C-terminal caspase recruitment domain (CARD) of caspase -4 and -5 in humans (caspase-11 in mice) resulting in the oligomerization and autoproteolysis of these caspases. Non-canonical activation does not require priming as caspase-4 is expressed at high levels in human cells. Following, the activated caspases cleave gasdermin d (GSDMD) directly to induce pyroptotic cell death through plasma membrane pores formed by the cleaved

N-terminal fragment of GSDMD. Moreover, the activation of these caspases leads to the opening of pannexin-1, a membrane channel that releases ATP. This extracellular ATP activates the purinergic P2X7 receptor leading ultimately to K^+ efflux, canonical NRLP3 activation and release of IL-1 and IL-18.(10,12,13,23–25). From this data, it can be gathered that the 'non-canonical inflammasome' not only can directly cause pyroptosis but can also activate the canonical NRLP3 inflammasome.(22)

2.2.3 Alternative activation of NLRP3

Differing from both the canonical and non-canonical pathways, the alternative pathway of NLRP3 activation does not initiate pyroptosis.(13) Given certain conditions, stimulation of human monocytes by LPS does not seem to depend on a second signal in order to activate caspase-1 -dependent Il-1β maturation and secretion. In this particular process an alternate caspase, caspase-8, a known enzyme for initiating apoptosis via the extrinsic pathway, elicits the NLRP3 activation through the TLR4-TRIF-RIPK1-FADD-Caspase-8 signaling while not requiring K^+ efflux or ASC speck formation. In murine dendritic cells, continuous exposure to LPS led to increased secretion of IL-1β independently of the purinergic P2X7 receptor. What is more to consider lies in the fact that in murine macrophages FADD and Caspase-8 are known contributors to NF-κB-dependent priming and post-transcriptional activation of the NLRP3 inflammasome suggesting that cross-interactions between apoptosis and pyroptosis pathways are yet to be assessed.(12,13,24) Over the past years, significant efforts have been dedicated to studying the precise mechanisms of NLRP3 inflammasome activation. Nevertheless, much remains to be done to fully

comprehend how various cell signaling events come together to trigger the activation of the NLRP3 complex.(12)

3. The role of inflammation in Type 2 Diabetes Mellitus (T2DM)

3.1 General scope of inflammation – Link between IL-1β, Il-18 and T2DM

Inflammation is generally portrayed as feedback to infectious and non-infectious injury, involving both cellular and humoral components of the immune system.(26) Inflammatory responses are indispensable for tissue restoration and survival and require the coordination of numerous complex signals across various cells and organs. However, exuberant and prolonged inflammatory activity facilitates the pathogenesis of multiple conditions, acute and chronic. This seems to be the case with metabolic disease with the latest research pointing that there is an undisputed linkage between inflammatory and metabolic signaling. Immune response and metabolic regulation are highly incorporated and the proper function of each is dependent on the other.(27)

A number of studies conclude that systemic, low-grade, sterile tissue inflammation has been acknowledged as the fundamental biological cornerstone of T2DM and its related complications.(2) This subclass of inflammation, often referred to as metabolic inflammation, is primarily driven by nutrient and metabolic surplus activating a similar array of molecules and signaling pathways as those involved in classical inflammation.(27) T2DM can be predicted by elevated levels of inflammatory proteins such as interleukins (IL-1β, IL-6, IL-18), chemoattractant

protein 1 (MCP1) and C-reactive protein (CRP).(21) Interleukin IL-1β stands out among the complex network of pro-inflammatory cytokines linked to chronic metabolic diseases. It has also been playing a pivotal role in initiating and perpetuating inflammation-induced organ dysfunction in DM.(2)

IL-1β is a master regulatory cytokine with potent pro-inflammatory capacity and is known to contribute to the inflammatory response in various metabolic diseases including diabetes.(21) More specifically, Il-1β is linked to inflammation in adipose tissue and insulin resistance.(28,29) Persistent elevation of IL-1β results in higher insulin levels, promoting glucose uptake and metabolism in macrophages, increasing insulin receptor expression, and intensifying their inflammatory state. Notably, IL-1β alone can enhance glucose uptake in macrophages.(8) IL-1β reduces tyrosine phosphorylation and gene expression of insulin receptor substrate-1 (IRS-1). This effect can occur through the activation of stress kinases, such as JNK and IKK, which instead promote serine phosphorylation of IRS-1. Serine phosphorylation, however, interferes with IRS-1's ability to transmit insulin signals. As a result, the downstream pathway of insulin signaling is disrupted and insulin resistance is induced.(30) Moreover, IL-1β stimulates the expression of tumor necrosis factor-alpha (TNFα), a cytokine that further exacerbates insulin resistance. Build-up of lymphocytes in adipocytes driven by IL-1β and IL-18, diminish tissue sensitivity to insulin to an even greater extent.(31)

IL-18, also known in the bibliography by the name of interferon gamma inducing factor, is a significant pro-inflammatory molecule with involvement in the activation process of inflammatory mediators and the regulation of the cytotoxic activity of natural killer (NK) cells and T cells.(32–34) It ,moreover, has assisting role in the pathogenesis of autoimmune and chronic inflammatory diseases, particularly in T2DM.(35) In support of that statement, Krogh-Madsen et al. study found that augmentation of IL-18 gene expression in muscle fibers is consistent with TNF-provoked insulin resistance, indicating the role of these molecules in T2DM manifestation.(36) Lindegaard et al. conducted experiments in murine species and established significant correlation between IL-18 and insulin resistance. In particular, IL-18 receptor knockout mice exhibited decline in their metabolic profile with high weight gain, lipid deposition and spike in inflammation markers. A hallmark finding was the reduced adenosine monophosphate activated protein kinase (AMPK) signaling in skeletal muscle tissue which under normal circumstances, activated by IL-18, enhances fat oxidation and reduces insulin resistance, indicating IL-18's involvement in metabolic homeostasis.(37) Esposito et al. showed that IL-18 levels in fasted people were higher to those diagnosed with T2DM. Moreover, a single high-fat meal was adequate to skyrocket the levels of IL-18 irrespective of T2DM diagnosis.(38) Thorand et al. demonstrated in a population-based human cohort study that elevated serum levels of IL-18 were significantly associated with an increased risk of T2DM, and this relationship was independent of CRP and IL-6 levels.(39) Not long ago, Zhuang et al. used a Mendelian randomization method to assess if there is causative relationship between Il-18 circulating levels and T2DM. The authors concluded that the pro-inflammatory actions of IL-18 in β-cell dysfunction is what aggregates the risk of T2DM.(40) Overall, these findings indicate that IL-18 triggers

inflammatory responses that could contribute to the development of insulin resistance and the onset of T2DM.

3.2 Obesity, Inflammation and Insulin resistance

Excessive calorie intake has resulted in a substantial rise in obesity, dyslipidemia and T2DM in today's world.(41) Adipose tissue has sparked quite significant attention as a primary organ contributing to obesity-induced insulin resistance.(42) For many years the scientific community thought of adipose tissue as an organ exclusively in charge of energy storage.(41) However, over the past two decades the search for a linkage between obesity and the pathogenesis of insulin resistance has led to the conclusion that excess nutrient consumption and immune system activation, particularly in organs responsible for energy homeostasis such as adipose tissue, are closely related. Data suggests that inflammation results from obesity and recent insights indicate that it has a causative role in producing insulin resistance either by impeding insulin secretion or by disrupting different aspects of energy homeostasis.(43) The discovery that tumor necrosis factor-α (TNF-α) is overexpressed in the adipose tissue of obese mice, marked the first definitive connection between obesity, diabetes, and chronic low-grade inflammation.(27) Randle et al. was among the first to shed light on the association between obesity and insulin resistance. According to this study increased free fatty acids (FFA) compete glucose for oxidative metabolism in insulin-sensitive tissues, exhibiting the insulin resistance.(44) More specifically, TNF-α has metabolic effects in peripheral tissues including elevation of free fatty acid (FFA) levels by altering the expression of genes

regulating lipolysis and lipogenesis. Increased FFA levels are linked to insulin resistance in skeletal muscle and enhanced hepatic gluconeogenesis. Additionally, elevated FFA levels are associated with early hypersecretion of insulin, which eventually results in impaired pancreatic insulin secretion capacity.(45)

3.2.1 Activation of the innate immune system in Obesity

Adipose cells formulate a heterogenous in composition tissue that comprises of mature adipocytes, immature adipocytes also called preadipocytes, endothelial cells, fibroblasts and other cells of the immune system.(46) A variety of fat depots have been recognized, each serving distinct physiological and metabolic function. In humans, subcutaneous fat is the largest fat depot accounting for approximately 70-80% of the body fat. The second in size fat depot is visceral fat which comprises 10-15% of total body fat.(44,47) Visceral fat, also known as abdominal or omental, is considered to be the metabolically 'unhealthy' fat depot. Adipocytes in the visceral fat depot exhibit significantly higher fatty acid fluxes compared to those within the superficial subcutaneous abdominal fat, showcasing a much less favorable inflammatory profile.(44,48) On top of that, visceral fat in comparison to abdominal subcutaneous fat, is marked by elevated production of proinflammatory cytokines as in tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and limited secretion of the anti-inflammatory adipokine, adiponectin.(49,50) Both visceral fat and deep subcutaneous fat have been linked to insulin resistance.(46)

It is now widely recognized, by numerous studies conducted in the last few years, that obesity induces a state of chronic low-grade inflammation, as evidenced by elevated production of cytokines and proinflammatory adipokines in adipose tissue.(46,51) However, it should be stressed that visceral adiposity in particular is accountable for the increased levels of TNF-α production in both the adipocyte and the macrophage within the adipose tissue.(52) Excess caloric intake relative to energy expenditure results in fat accumulation. When fat is stored predominantly in the superficial subcutaneous adipose tissue depot, the inflammatory response is usually minimal or absent. However, if fat accumulation shifts towards abdominal fat and ectopic depots, a persistent low-grade inflammatory response is likely to occur.(46) Buildup of abdominal fat triggers inflammation through various mechanisms. Redundant caloric consumption expands adipose tissue and causes adipocyte hypertrophy. It has been speculated that, as a defense mechanism to obesity, the body produces local hypoxia and adipocyte apoptosis which fundamentally invokes signals to mobilize macrophages.(4,53) Hypertrophic adipose tissue begins to excrete TNF-α initially at low levels, stimulating preadipocytes and endothelial cells to produce the monocyte chemotactic protein-1 (MCP-1), also known as CCL2. Augmented expression of chemokines like CCL2 promote the infiltration of inflammatory cells, initiating a vicious circle of chronic inflammation.(41,54) Adding to the proinflammatory properties of local hypoxia, during adipose tissue expansion the rate of protein synthesis rises leading to aggregation of misfolded proteins and thus to endoplasmic reticulum (ER) stress which further enhances inflammation.(55–57)

Macrophages experience dramatic transformations during obesity.(43) Aside from adipocyte hypertrophy, obesity provokes changes in the stromovascular composition

of cells as well, shifting their phenotype towards inflammatory state.(58) Macrophages may present pro- or anti- inflammatory properties within the adipose tissue depending on their activation status.(59) M1 macrophages, activated by TNF-α and lipopolysaccharides (LPS), are proinflammatory whereas M2 macrophages, which are activated by interleukins -4 and -10 (IL-4 and IL-10), appear to manifest anti-inflammatory potency. Adipose tissue inflammation resulting from obesity increases the ratio of M1 to M2 and predisposes to metabolic disease and insulin resistance.(43,60)

3.2.2 Inflammation and Insulin Resistance

Insulin resistance can be defined as a state characterized by reduced sensitivity of insulin target cells, such as skeletal muscle, adipose tissue, hepatic cells, to insulin, resulting in diminished ability of insulin to regulate key metabolic actions.(46,61) There is now substantial evidence linking inflammation directly to insulin resistance. Studies on obese knockout mice with inactivated inflammatory signaling have shown interruption of the relationship between obesity-induced inflammation and insulin resistance, either by directly blocking insulin action or by attenuating insulin signaling in target tissues.(43)

Insulin and insulin-like growth factor (IGF) receptors are part of a tyrosine kinase family that coordinate their signaling via docking proteins. Among the many intracellular substrates utilized by the above-mentioned receptors, six belong to the family of insulin receptor substrate (IRS) proteins. A definitive step in the insulin signaling cascade is the phosphorylation of IRS proteins, which is stimulated by

insulin itself.(27) When insulin binds to its receptor, it triggers autophosphorylation of tyrosine residues on the receptor's intracellular domain. In the metabolic pathway of insulin signaling, the insulin receptor substrate (IRS) docks the insulin receptor and undergoes tyrosine phosphorylation through the kinase activity of the phosphorylated insulin receptor. The signal transduction proceeds with the recruitment of phosphatidylinositol-3 kinase (PI3K) and Akt/protein kinase B (PKB) which when activated induces downstream effects.(62) The metabolic pathway of insulin signaling is transmitted via PI3 kinase and affects primarily GLUT-4 translocation and hormone-sensitive lipase inhibition whereas the other basic pathway involves signal transduction via the renin-angiotensin system/mitogen-activated protein (Ras/MAP) kinase and has mitogenic properties.(46)

The phosphorylation of IRS proteins, in insulin-receptor signaling, is the most common defective step of systemic insulin resistance, documented in experiments regarding both animal models and humans. It is now well-documented that when IRS proteins are phosphorylated at serine residues by various kinases, their ability to engage to insulin signaling gets disrupted.(27) This particular mechanism is notably impaired in obesity and is thought to be fundamental to the development of obesity-related insulin resistance.(63) Proinflammatory cytokines TNF-α, IL-6, IL-1 and non-esterified fatty acids (NEFA), which as stated before, are all crucially involved in obesity-associated low-grade inflammation, can directly hinder insulin signaling in healthy humans leading to insulin resistance.(46) More specifically, these molecules utilize numerous intracellular serine/threonine kinases including c-Jun NH_2-terminal kinase (JNK), protein kinase C (PKC), inhibitor of κappa-B kinase (IKKb) and mTOR which promote insulin resistance through phosphorylation of IRS-1 on its serine

residues.(62–64) A different molecular link between inflammation and suboptimal insulin action include the suppressor of cytokine signaling (SOCS) proteins 1 and 3 and nitric oxide (NO). SOCS proteins are stimulated during inflammatory response by IL-6 and adipokines and lead to the degradation of insulin receptor substrates (IRS) through ubiquitination. The expression of SOCS-3 in particular, is dramatically augmented in patients with insulin resistance and T2DM. The degradation of IRS is, also, associated with NO, a molecule produced by nitric oxide synthase (iNOS) and induced by cytokines. The activity of iNOS leads to a reduction in PI3K/AKT activity, the primary mediator of IRS signaling, thereby inhibiting the insulin signaling pathway.(4,27,44)

3.3 Inflammation, Endothelial dysfunction and Cardiovascular disease in T2DM

Oxidative stress, inflammation, and endothelial dysfunction are interconnected elements within an etiological framework associated with the development of cardiovascular disease (CVD), which have also been implicated in the pathogenesis of insulin resistance and T2DM.(65,66) Endothelial dysfunction emerges early in the progression of cardiovascular disease and represents one of the initial manifestations of both T2DM and cardiovascular conditions. This dysfunction is marked by the dysregulated secretion of various inflammatory mediators, such as IL-1β, TNFα, histamine, and bradykinin, which compromise inter-endothelial junction integrity.(67,68)

In diabetes mellitus (DM), the vascular endothelium plays a crucial role in regulating inflammatory progression and maintaining cardiovascular homeostasis as a dynamic and adaptive interface. As stated before, proinflammatory cytokines,

including TNF-α, IL-1β, and IL-6, significantly contribute to endothelial cell injury. TNF-α induces cytotoxic effects, IL-6 increases endothelial permeability, and IL-1β promotes nitric oxide synthase expression while also exerting a synergistic effect with TNF-α.(69) Nitric oxide (NO), a key mediator produced by endothelial cells, plays a vital role due to its vasodilatory, antiplatelet, antiproliferative, anti-inflammatory, antioxidant, and permeability-reducing properties. NO also inhibits leukocyte rolling and adhesion while suppressing the cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1).(70) Endothelial dysfunction is linked to reduced nitric oxide (NO) availability, either due to impaired NO production or diminished biological activity. Oxidative stress further contributes to this decline by inhibiting NO synthesis in affected cells.(61) In patients with poorly controlled diabetes, elevated oxidative stress is primarily driven by hyperglycemia. This occurs through five metabolic pathways: increased glucose flux via the polyol pathway, heightened formation of advanced glycation end products (AGEs) and their receptors, activation of protein kinase C isoforms (β, δ, and α), excessive activity of the hexosamine pathway, and a reduction in antioxidant defenses.(71) Brownlee et al. identified a unifying link among these mechanisms which is the excessive production of superoxide (O_2^-) by the mitochondrial electron transport chain, leading to a generalized state of oxidative stress. Superoxide can interact with nitric oxide to form peroxynitrite and nitrotyrosine, which contribute to endothelial injury and further vascular damage.(72)

Ultimately, the inflammatory state associated with diabetes enhances platelet hyperreactivity and adhesion to the endothelium, fostering a pro-thrombotic phenotype and elevating the risk of cardiovascular mortality.(73) Diabetes mellitus can increase platelet susceptibility to activation through various mechanisms. Several

metabolic and cellular abnormalities have been implicated in enhancing platelet reactivity in diabetic patients. Notably, hyperglycemia contributes to heightened platelet activity by glycosylating platelet surface proteins, activating protein kinase C, inducing P-selectin expression, and altering osmotic balance, all of which promote platelet activation and adhesion further promoting atherothrombosis.(74)

4. The role of NLRP3 inflammasome in the development of type 2 Diabetes Mellitus and its macrovascular complications

4.1 Regulation of T2DM by NRLP3

The NLRP3 inflammasome appears to function as a detector of metabolic danger signals, such as endogenous DAMPs and PAMPs, which accumulate in obesity. These signals include saturated free fatty acids (FFAs), ceramides, elevated glucose levels, uric acid, and Islet Amyloid Polypeptide (IAAP). The activation of the NLRP3 inflammasome and the subsequent production of IL-1β were initially observed in pancreatic β-cells and macrophages infiltrating the islets. This process leads to IL-1β secretion, triggering the release of various cytokines and chemokines.(21,75) Inflammation resulting from nutrient excess and obesity is becoming widely acknowledged in obesity-related conditions, including T2DM. Studies demonstrate that caloric restriction and intermittent fasting can counteract nutrient overload while simultaneously lowering inflammation.(58) Inflammation that stems from obesity incorporates both the innate and adaptive immune systems. Furthermore, obesity activates inflammatory cells within various lipid-rich organs, leading to the release of cytokines and acute-phase proteins. The activation of pro-inflammatory pathways is

driven by an excess of nutrient byproducts, such as saturated fatty acids, ceramides, and elevated glucose levels, which interact with pattern recognition receptors on leukocyte and adipocyte membranes. Overall, human studies demonstrate that insulin resistance and obesity are closely linked to increased NLRP3 expression in adipose tissue.(21,76) Experiments conducted on murine models validate this finding. A high-fat diet (HFD) enhances NLRP3 expression in murine adipose tissue, whereas a calorie-restricted diet decreases its expression. Consequently, blocking NLRP3 in mice safeguards against HFD-induced obesity and insulin resistance.(77) Various immune cells, particularly pro-inflammatory macrophages, influence adipose tissue balance by increasing the production of cytokines like TNF, IL-1β, and IL-6. Activation of the NLRP3 inflammasome appears to play a crucial role in adipocyte differentiation, promoting a shift towards greater insulin resistance in adipocytes. Consistently, weight loss through caloric restriction and exercise in obese individuals with type 2 diabetes (T2DM) leads to a reduction in NLRP3 expression.(21,78)

The NLRP3 inflammasome has been widely studied for its pivotal relation to inflammation and the progression of T2DM. Liu et al. examined the critical role of NLRP3 in the pathogenesis of T2DM. They showed that IL-1β levels are elevated in T2DM patients, indicating a potential link between IL-1β and the development of T2DM.(79) Overabundant IL-1β production in T2DM leads to several consequences. First and foremost, it triggers the expression of other inflammatory mediators (such as IL-18, IL-33) through IL-1 receptor signaling, which amplifies the inflammatory response. Evidence shows that chronic upregulation of IL-1β leads to elevated insulin levels, which may negatively impact metabolism. This could be due to insulin's role in amplifying the inflammatory state of macrophages by promoting glucose uptake and metabolism, as well as increasing the expression of insulin receptors in macrophages

of diet-induced obesity (DIO) mice. IL-1β has been shown to enhance glucose uptake into macrophages, with insulin amplifying its pro-inflammatory effects by regulating the insulin receptor, glucose metabolism, and reactive oxygen species production. NLRP3 inflammasome play a key role in mediating IL-1β secretion. Increasing glucose excretion through urine can help prevent tissue glucose overload, thereby mitigating the harmful effects of glucose-induced IL-1β.(21,80) Secondly, IL-1β induces oxidative stress and endoplasmic reticulum (ER) stress, both of which are closely associated with T2DM and last but not least, it activates c-Jun N-terminal kinases (JNKs), leading to serine phosphorylation of insulin receptor substrate 1 (IRS-1) with subsequent reduction in the activity of the insulin-PI3K/AKT signaling pathway in insulin-sensitive tissues and ultimately, in insulin resistance.(21)

The role of the NLRP3 inflammasome in metabolic syndrome and T2DM can be divided into two main categories. The first category involves direct roles mediated by the detection of endogenous inflammasome activators, while the second focuses on indirect roles related to inflammasome-induced changes, which affect the gut microbiota. It is important to note that T2DM was the first metabolic disorder identified to be associated with NLRP3.(81,82) **(Table 1)**

Increased plasma free fatty acids (FFAs), primarily due to high-fat diet consumption, play a key role in the development of T2DM. Recent studies have identified a link between NLRP3 inflammasomes and lipid species associated with metabolic diseases, including saturated fatty acids (SFAs) and ceramides.(91,92) Palmitic acid, a prevalent free fatty acid (FFA), activates the NLRP3 inflammasome by inducing mitochondrial ROS production and lysosomal destabilization, thereby contributing to insulin resistance.(21) Palmitate-triggered NLRP3 inflammasome activation compromises endothelial tight junctions, contributing to endothelial

damage in obesity. In response to a high-cholesterol diet, intestinal epithelial cells activate caspase-1 following IL-1β-dependent myeloid cell accumulation in the intestine. Additionally, in monocytes, palmitate stimulates caspase-4/5 activation, leading to the release of IL-1β and IL-18.(93) Elevated levels of saturated fatty acids (SFAs) drive the production of ceramides, a lipid species linked to inflammation in obesity-induced diabetes. Ceramides have been shown to activate the NLRP3 inflammasome in cultured macrophages and adipose tissue explants from diet-induced obese mice. Exposure to ceramides triggers NLRP3-dependent caspase-1 activation, further amplifying the inflammatory response.(94)

Hyperglycemia is a key characteristic of T2DM. For nearly two decades, it has been established that the NLRP3 inflammasome is activated in response to elevated glucose levels. Glucose has been shown to activate protein kinase C alpha (PKCα), which, through phosphorylation of p38, MAPK, and extracellular signal-regulated kinases 1/2 (ERK1/2), triggers NF-κB activation and subsequent IL-1β transcription in monocytes, thereby preparing the cells for inflammasome activation.(95) High glucose concentrations can provide the priming signal for IL-1β transcription by activating thioredoxin-interacting protein (TXNIP), which subsequently enhances IL-1β expression. Additionally, elevated glucose levels stimulate reactive oxygen species (ROS) production, serving as the second signal that triggers inflammasome activation, caspase-1 activation, and IL-1β processing in pancreatic islets.(96,97) TXNIP plays a vital role in regulating lipid and glucose metabolism through a range of actions, including the modulation of β cell function, peripheral glucose uptake, adipogenesis, hepatic glucose production, and substrate utilization. In animal models, upregulation of TXNIP has been shown to induce pancreatic β cell apoptosis and decrease insulin sensitivity in peripheral tissues such as skeletal muscle and adipose tissue. In contrast,

animals lacking TXNIP are protected from diet-induced insulin resistance and the development of type 2 diabetes.(98)

Intestinal microflora is essential for metabolic, immune, and protective functions. Disruptions in the composition of the gut microbiota, a condition known as dysbiosis, have been linked to the development of inflammatory diseases like T2DM. Inflammasomes play a crucial role in regulating gut microbiota composition, as demonstrated by recent studies in mouse models. These studies have shown that the absence of inflammasome components increases susceptibility to colitis and tumorigenesis, which is associated with dysbiosis. Growing evidence underscores that inflammasome structure is vital for maintaining intestinal epithelial integrity and defending against pathogenic threats. However, the specific mechanisms and factors driving inflammasome activation by intestinal microbiota remain unclear.(99,100)

4.2 NLRP3 and Diabetic Macrovascular Disease

As stated previously in this review, endothelial dysfunction is etiologically interconnected with T2DM and its subsequent CVD risk.(Table 2) For that reason, it is deemed appropriate to examine this basic constituent more thoroughly.

Endothelial dysfunction is presumed to be the first key step to activating the NLRP3 inflammasome in T2DM.(21) Endothelial cells form a single-layer lining on the inner surface of blood vessels and serve multiple essential functions. They facilitate the transport of nutrients, including glucose, as well as hormones and macromolecules, from the bloodstream to surrounding tissues, supporting their growth and metabolic processes.(113) Endothelial cells play a crucial role in maintaining vascular function by regulating cell adhesion, vessel wall integrity,

permeability, thrombus formation, and fibrinolysis. They control blood flow, ensure fluidity, facilitate leukocyte trafficking, support angiogenesis, and contribute to immune responses.(114) Additionally, they secrete mediators that modulate vascular tone, including vasoconstrictors such as endothelin-1 (ET-1) and thromboxane A2, as well as vasodilators like nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor. Acting as a barrier between blood and tissues, they are fundamental to the body's inflammatory response.(115,116) Endothelial cells serve as a frontline defense against endogenous molecules and microbes that trigger infections or inflammation. Through their innate immune system receptors, they can recognize a wide range of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). A compromised endothelium is characterized by phenotypic alterations, inflammation, and disrupted permeability, among other dysfunctions.(117) Furthermore, endothelial dysfunction is marked by impaired vasodilation, heightened pro-thrombotic and pro-inflammatory activity, and an increased redox state. In diabetes, it represents an early stage of various hyperglycemia-related vascular diseases, such as atherosclerosis, which drive vascular inflammation and eventually lead to atherosclerotic lesions. This dysfunction emerges in the initial stages T2DM through a complex interplay of altered cell signaling, elevated oxidative stress, pro-inflammatory activation, and mitochondrial dysfunction.(118) The progression of hyperglycemia-induced vascular endothelial inflammation compromises the endothelial barrier, ultimately contributing to diabetes-related vasculopathy. As a result, vascular complications are the leading cause of mortality and disability in individuals with diabetes.(119,120)

Diabetes mellitus (DM) is a major risk factor for cardiovascular disease, increasing the likelihood of stroke or heart attack (major adverse cardiovascular

events—MACE) by two to three times, primarily due to accelerated atherosclerosis.(2) NLRP3 is well recognized for its critical role in the initiation and progression of atherosclerosis by driving vascular inflammation. Atherosclerotic plaques exhibit high mRNA and protein expression of NLRP3, ASC, caspase-1, IL-1β, and IL-18 within macrophages, foam cells, and endothelial cells. Numerous studies have linked inflammasome-derived IL-1β and IL-18 to the pathogenesis of atherosclerosis.(121) NLRP3 is linked to cholesterol crystals both within and outside macrophages and foam cells. In fact, cholesterol plays a key role in activating NLRP3 during atherogenesis. An in vitro study by Duewell et al. demonstrated that cholesterol crystals are taken up by phagocytes, triggering NLRP3 activation through a mechanism involving phagolysosomal damage.(109)

In a groundbreaking study, Lee et al. established the foundation for exploring the role of NLRP3 in diabetes mellitus (DM)-associated cardiovascular disease (CVD). Their findings revealed an upregulation of inflammasome components NLRP3 and ASC in monocytes from newly diagnosed, untreated type 2 DM patients, accompanied by heightened basal and inducible inflammasome activation in response to DAMP signals. Furthermore, drug-naïve type 2 DM individuals exhibited significantly elevated serum levels of IL-1β and IL-18 compared to healthy controls. Notably, in vitro knockdown of NLRP3 in monocytes from DM patients abolished the ability of metabolic DAMPs to trigger IL-1β and IL-18 secretion. Consistent with these findings, a preclinical study in a rat model of type 2 DM demonstrated that excessive NLRP3 activation and pyroptosis were closely linked to pathological structural and functional alterations in the heart, which were effectively reversed by NLRP3 silencing.(103,104) Experimental studies using animal and cellular models of atherosclerosis have provided mechanistic insights into inflammasome activation in

diabetic macrovascular complications. NLRP3 has been implicated in hyperglycemia-induced endothelial inflammation in both in vitro and in vivo settings. In human umbilical vein endothelial cells and atherosclerotic plaques of diabetic mice, high glucose levels led to the overexpression of adhesion molecules, a process that was effectively inhibited by NLRP3 knockdown and IL-1 receptor antagonism.(105) In addition to glucose toxicity, dysregulated lipid metabolism in diabetic blood vessels may also contribute to inflammasome activation. In a porcine model of atherosclerosis and DM, elevated aortic levels of sterol regulatory element-binding protein (SREBP)-1 were correlated with increased expression of NLRP3 inflammasome components. Strong immunostaining for this lipogenic transcription factor was detected in macrophages and endothelial cells within early-stage lesions (fatty streaks) as well as in the fibrous cap and cholesterol crystals of advanced atherosclerotic plaques. Notably, similar patterns were observed in aortic biopsy samples from human patients with atherosclerosis and DM.(108)

Additionally, there are more parameters linking NLRP3 inflammasome with vascular health as research suggests that NLRP3 activity can be influenced by mechanical forces, such as hemodynamic stress, which impact endothelial cell responses. Oscillatory shear stress has been identified as a novel regulator of endothelial inflammasome activation in atherogenesis, acting through the downregulation of forkhead box P transcription factor 1 (Foxp1) by inhibiting Kruppel-like factor 2 expression in endothelial cells. Consistently, Foxp1 expression was found to be markedly reduced in both human and mouse coronary atherosclerotic endothelium. Additionally, mitochondrial DNA has been implicated in diabetes-associated endothelial dysfunction and vascular inflammation by activating NLRP3

through mechanisms involving calcium influx and reactive oxygen species (ROS) production.(122)

As stated before, compelling evidence supports the bidirectional relationship between glucose metabolism disorders and stroke events. Insulin resistance serves as both an early predictor and an independent risk factor for cardiovascular and cerebrovascular events. For that reason, targeting the assembly and activity of the NLRP3 inflammasome could serve as a promising and innovative therapeutic approach for cerebrovascular ischemic disease associated with insulin resistance.(123)

Similar to coronary artery disease (CAD) and cerebrovascular disease (CeVD), peripheral artery disease (PAD) is primarily a consequence of atherosclerosis.(124) Cai H. et al., in a study among diabetic patients with and without PAD, showed that the expression of the NLRP3 inflammasome components (NLRP3, ASC, caspase-1) was markedly elevated in the arteries of patients with PAD, suggesting that NLRP3 inflammasome activation may play a role in the pathological progression of the disease. However, the endogenous expression of the NLRP3 inflammasome pathway in diabetic peripheral artery disease is still unclear.(125)

Regrettably, apart from cardiovascular disease, research into the molecular mechanisms, particularly the pathogenic processes involving the inflammasome, has been somewhat slower compared to studies on other diabetes-related macrovascular complications. To date, only a limited number of articles have explored the role of NLRP3 in the pathophysiological changes and clinical manifestations of diabetic cerebrovascular disease and peripheral artery disease in literature.

5. Therapeutic strategies

The established correlation between the NLRP3 inflammasome and various inflammatory , metabolic and cardiovascular diseases has sparked intense scientific interest in discovering effective therapies targeting the NLRP3 inflammasome. The key role of NLRP3 inflammasome in Type 2 Diabetes Mellitus and CVDs in particular, has made the NRLP3 an attractive target for pharmaceutical innovation, leading to the development of molecules capable of inhibiting the NLRP3. To this date, several NLRP3 inflammasome inhibitors are in preclinical and clinical phase.(9,126) NLRP3 inflammasome inhibition can be achieved through multiple targets by leveraging its intricate signaling cascade. While some of these specifically target and block the NLRP3 inflammasome, others exert broader effects that indirectly lead to the inhibition of NLRP3-mediated signaling.(16) It has been demonstrated that targeting inflammasome-related pathways or molecules effectively treats insulin resistance (IR) and reduces the onset and progression of T2DM.(127) We will highlight NLRP3 associated therapies that have been tested in cardiovascular and metabolic diseases.(Table 3)

5.1 Potential approaches for pharmacological inhibition of the NRLP3 inflammasome

With the discovery of inflammasome stimuli in the adipose tissue of obese subjects , various endogenous molecules and chemical compounds have been found to directly suppress the NLRP3 inflammasome by targeting priming, activation, or ASC oligomerization. Some of these are now being translated from experimental research

into promising clinical applications.(128) Various inhibitor targets include disrupting NLRP3–NLRP3 and NLRP3–ASC interactions, blocking the ATP-binding domain to inhibit ATPase activity, preventing NLRP3 post-translational modifications, inhibiting caspase-1, suppressing NT-GSDMD pore formation, and neutralizing IL-1β and IL-18.(9)

5.1.1 NLRP3 inhibitors

The benefit of targeting the core components of the NLRP3 inflammasome is the prevention of pyroptosis, an effect independent of IL-1β or IL-18 inhibition. The effectiveness of NLRP3 inflammasome inhibitors has been evaluated both in vitro, by stimulating cells with lipopolysaccharide (LPS) and ATP (or nigericin, cholesterol crystals, and monosodium urate crystals) and in vivo.(9) Most of the compounds discussed here have been tested in animal models of cardiovascular disease**.(Figure 1)**

5.1.1.1 MCC950

MCC950, also known in the bibliography as CP-456,773 or CRID3, was described for the first time in 2001 for its ability to inhibit IL-1β processing. It was later on further characterized by Coll et al. as a potent inhibitor of the NLRP3 inflammasome both in vivo and in vitro.(129) MCC950 is a small diarylsulfonylurea-containing molecule that binds with non-covalent bonds directly to the NACHT domain of NLRP3 near the Walker B motif, which is necessary for the inflammasome assembly, consequently suppressing ATP hydrolysis and therefore preventing ASC oligomerization and IL-1β release.(9,130) MCC950 specifically inhibits canonical and non-canonical NLRP3 inflammasome activation in both mouse and human

macrophages in vitro.(131)MCC950 is a selective inhibitor of the NRLP3 inflammasome, lacking inhibitory properties against NLRP1, NLRC4 and AIM2 inflammasomes.(132) Due to its high potency and specificity in inhibiting the NLRP3 inflammasome, MCC950 has been widely used as a prototypical inhibitor and a research tool to explore the mechanisms of NLRP3 inhibition and its role in disease pathogenesis.(24) It has been studied in many different mouse models for DM-accelerated atherosclerosis, metabolic and inflammatory disease in general. It was shown that MCC950 reduces atherosclerotic plaque development, decreases the expression of adhesion molecules within the plaque, and lowers the number of macrophages present in the plaque.(9) Multiple studies have demonstrated that MCC950 can alleviate inflammation, enhance vascular function, and prevent diabetes-related atherosclerosis in streptozotocin-induced $ApoE^{-/-}$ mice.(73) Additionally, in mice fed a high-fat, high-cholesterol diet or treated with angiotensin II, MCC950 prevented aortic dilation, as well as the dissection and rupture of aortic segments in the thoracic and abdominal regions.(16) In mice with permanent coronary artery occlusion, MCC950 (10 mg/kg) decreased inflammatory cell infiltration, caspase-1 activation, and levels of IL-18 and IL-1β and also, reduced myocardial fibrosis, thus promoting improved cardiac remodeling.(9) In a mouse model of postmenopausal heart disease, an 8-week administration of MCC950 (10 mg/kg, three times per week) mitigated hypertrophic remodeling, enhanced systolic and diastolic function, and lowered atrial natriuretic peptide (ANP) and BNP mRNA levels.(16,133) However, the clinical development MCC950 was discontinued due to increased renal inflammation and hepatotoxicity.(130,134)

5.1.1.2 Glyburide derivates

Glyburide, also known as glibencamide is a sulfonylurea drug approved for treating Type 2 Diabetes Mellitus.(135) It blocks the ATP-sensitive potassium channel in pancreatic beta cells, facilitating insulin release.(13,136) Glyburide was the first identified compound to showcase selective inhibition of the NLRP3 inflammasome in bone marrow-derived macrophages stimulated with LPS/ATP, uncapable of affecting other inflammasomes (NLRP1, NLRC4, AIM2).(9) When tested against stimuli that function independently of the P2X7 receptor but rely on TLR4 signaling, glyburide effectively inhibited caspase-1 activation and IL-1β secretion, indicating that it acts downstream of the P2X7 receptor.(137) Although glyburide has demonstrated inhibitory effects against NLRP3 activation both in vitro and in vivo, the high dosage that is mandatory for its anti-inflammasome effect limit the use of it as anti-inflammatory drug.(8) . The cyclohexylurea moiety, which is involved in insulin release is not necessary for the inhibitory activity of the NLRP3 inflammasome. For this reason, a glyburide derivative named 16673-34-0 was developed, lacking the cyclohexylurea moiety while maintaining its inhibitory activity against NLRP3.(9,16) The effects of 16673-34-0 administration were evaluated in various cardiac injury models. When administered at a dose of 100 mg/kg, it suppressed cardiac caspase-1 activity and minimized infarct size in mice undergoing myocardial ischemia followed by 24 hours of reperfusion. Moreover, in mice treated with the cardiotoxic chemotherapy drug doxorubicin, intraperitoneal administration of 16673-34-0 enhanced cardiac function and decreased interstitial fibrosis. Equally beneficial results were obtained even when 16673-34-0 was administered 60 minutes after reperfusion. Based on 16673-34-0, innovative compounds were developed, one

them being JC-124 (N-Me sulfonamide analog of 16673-34-0).(9,16,138) In mice subjected to ischemia (30 or 75 minutes) and reperfusion (24 hours), intraperitoneal administration of 30 mg/kg of JC-124 effectively reduced infarct size and plasma troponin I levels.(9)

The precise mechanism of action of 16673-34-0 remains unclear. However, its efficacy downstream of multiple stimuli, combined with its lack of effect on AIM2 and NLRC4 inflammasome formation, suggests that it inhibits NLRP3, prevents NLRP3 conformational changes, and interferes with the interaction between NLRP3 and ASC.(138)

5.1.1.3 Bay 11-7082

Bay 11-7082 is an artificial kappa B kinase β (IKKβ) inhibitor with structural similarities to vinyl sulfone. It manifests its inhibitory properties by alkylating the cysteine residues in the NLRP3 ATPase region, leading to NF-κB pathway inhibition. However, apart from its IKKβ inhibitory activity, Bay 11-7082 can selectively block the NLRP3 inflammasome without impacting other inflammasome receptors such as NLRP1 and NLRC4.(139) In mouse models of experimental myocardial ischemia-reperfusion, administering Bay 11-7082 10 minutes prior to coronary artery reperfusion decreases leukocyte infiltration in the infarcted area and enhances cardiomyocyte survival, reducing infarct size. Comparable effects were seen in diabetic rats, where Bay 11-7082 mitigated myocardial injury following ischemia-reperfusion by decreasing pyroptotic cell death, NLRP3 inflammasome activation, and the expression of caspase-1 and IL-1β.(140) However, since Bay 11-7082 inhibits

both IKKβ and NLRP3, it is uncertain whether its beneficial effects result from the inhibition of NF-κB-dependent signaling or the blockade of the NLRP3 inflammasome.(16)

5.1.1.4 OLT1177

OLT117 is a small, beta-sulfonyl nitrile molecule with great bioavailability when taken orally that specifically inhibits NLRP3. It mitigates the release of IL-18 and IL-1β without impacting other inflammasomes (NLRC4 and AIM2) making it a selective NLRP3 inhibitor. OLT1177 prevents NLRP3 oligomerization, disrupting the interaction between NLRP3 and ASC and blocking the activation of the downstream signaling cascade. Additionally, OLT1177 directly binds to NLRP3 and inhibits its ATPase activity.(141) In an animal model of myocardial ischemia-reperfusion, OLT1177 effectively led to a dose-dependent reduction in infarct size and helped maintain cardiac function at both 24 hours and 7 days after reperfusion. It also improved ventricular function in a model of permanent coronary artery occlusion. Notably, the same study highlighted its clinical relevance, demonstrating that OLT1177 remained effective even when administered 60 minutes post-reperfusion. Overall, OLT1177 is a promising therapeutic candidate for NLRP3-related conditions, including heart failure (HF) and acute myocardial infarction (AMI).(16,142) Additionally, in a phase-1B pilot double-blind study involving patients with heart failure with reduced ejection fraction (HFrEF), OLT1177 was found to be safe. At the highest dose tested, it was linked to an improvement in left ventricular ejection fraction and increased treadmill exercise time after 14 days.(143)

5.1.1.5 Colchicine

Colchicine is a tricyclic alkaloid with therapeutic approval as a drug for treating inflammatory diseases such as gout and familial Mediterranean fever. It is also used off-label for the treatment of acute and recurrent pericarditis.(144) Colchicine interferes with the NLRP3 complex by disrupting microtubule action. Furthermore, colchicine suppresses neutrophil chemotaxis and diapedesis, potentially by stimulating the hepatic production of growth differentiation factor 15 (GDF-15), without directly affecting leukocyte function.(145) Recent studies have demonstrated that colchicine inhibits NLRP3 inflammasome formation in two ways. Firstly, by preventing P2X7-mediated pore formation and secondly, by disrupting the intracellular transport and spatial arrangement of NLRP3 and ASC, preventing their oligomerization.(146) In a mouse model of permanent cardiac ligation, colchicine administered at 1 mg/kg/day for 7 days enhanced survival and preserved left ventricular ejection fraction 4 weeks post-surgery. It also decreased the infiltration of neutrophils and macrophages, as well as the mRNA expression of pro-inflammatory cytokines and NLRP3 inflammasome components 24 hours after myocardial infarction.(147) Numerous clinical trials have demonstrated safety and efficacy of colchicine at low dosages (0.5 mg/day). In COLCOT study (Colchicine Cardiovascular Outcomes Trial), patients that suffered from acute myocardial infarction were given the same dose of colchicine (0.5 mg per day), had drastically lower risk of acute coronary syndromes compared to placebo at 22 months follow-up.(148) A different study, LoDoCo (Low-Dose Colchicine) assessed patients with stable coronary disease in medication regimen with colchicine and secondary prevention therapies such as statins. It was profoundly shown that colchicine lowered the risk of cardiovascular events. Additionally, colchicine was able to alter the

composition of coronary plaques and decrease the levels of high sensitive C-reactive protein (hsCRP) post-acute coronary syndromes. In murine models, experiments highlighted the impact of colchicine in reducing pericardial effusion in acute pericarditis.(9)

5.1.1.6 CY-09

CY-09, a newly introduced NLRP3 inhibitor, is an analog of cystic fibrosis transmembrane conductance regulator-172 (CFTR-172) without CFTR inhibitory activity.(13) It inhibits the NLRP3 complex by binding directly to the ATP-binding motif of the NACHT domain. Its medicinal effectiveness has been tested on murine models of type 2 Diabetes Mellitus. Particularly, in a mouse model of diabetic stroke, the administration of CY-09 was effective in preventing cardiac dysfunction linked to diabetic ischemic stroke.(149)

5.1.1.7 Tranilast

Tranilast (N-[30 -40 -dimethoxycinnamonyl]-anthranilic acid) is an analog of tryptophan metabolite with clinical approval for the treatment of numerous allergic disorders by reducing collagen synthesis. It is a newly recognized NLRP3 inflammasome inhibitor.(9) Tranilast specifically blocks NLRP3 activity without affecting NLRC4 or AIM2. When bonded to the NACHT domain of the NLRP3, Tranilast abolishes the direct NLRP3-NLRP3 and NLRP3–ASC interaction. Unable to oligomerize, NLRP3 loses its ATPase activity. Its inhibitory action remains unaffected

by upstream signaling pathways, including ROS production, ion efflux or mitochondrial damage.(150) Tested in two different murine models of atherosclerosis, Tranilast enhanced NLRP3 ubiquitination, restricting NLRP3 inflammasome assembly and thereby reducing the initiation and progression of atherosclerotic plaques.(137) More importantly Tranilast has been evaluated in multiple clinical trials, where it demonstrated safety and good tolerance even at high doses in patients.

5.1.1.8 INF4E

INF4E [ethyl 2-((2- chlorophenyl)(hydroxy)methyl)acrylate] is a synthetic alpha,beta-unsuturated carbonyl- or cyano- derivative with anti-pyroptotic properties. INF4E has the ability to inhibit the NLRP3 ATPase activity disrupting the activation process of caspase-1.(151) In murine models of myocardial ischemia, pretreatment with INF4E reduced infarct size and improved left ventricular pressure. Additionally, INF4E administration in these specimens limited the expression of the NLRP3 components, improved mitochondrial function and engaged the protective reperfusion injury salvage kinase (RISK) pathway.(152) However, INF4E demonstrated cytotoxic properties. For this reason, new compounds that share the reactive Michael acceptor moiety of INF4E and a sulfonamide/sulfonylurea part were developed. Among these compounds, INF58 shows the most promise, however, its potential as a cardio-protective agent has not yet been evaluated.(9)

5.1.1.9 Hydrogen Sulfide

Endogenous Hydrogen Sulfide (H_2S) is a gasotransmitter that demonstrates a pivotal physiological role.(16) H_2S is a molecule with a broad range of biological activities. Apart from its importance in myocardial ischemia it exerts antioxidative, anti-apoptotic and anti-inflammatory properties. Experimental models of acute myocardial infarction validate that H_2S donors reduce damage to cardiomyocytes.(153) Particularly, the H2S donor Na_2S that was tested in murine specimen undergoing ischemia-reperfusion injury, led to minimization of the infract size primarily due to reduced NLRP3-dependent caspase-1 activation.(154) NaHS, another H_2S donor, diminished the IKKβ/NF-κB signaling pathway introducing cardioprotective properties in a hemorrhagic shock model. A study on macrophages brought to light that H_2S donor Sodium Thiosulfate inhibits NLRP3 activation signals. Consequently, H_2S appears to attenuate inflammasome activity by acting both on the priming and trigger signals.(9)

5.1.2 Diabetic Medication and NLRP3 modulation

A variety of well-established anti-diabetic drugs have demonstrated significant impact on NLRP3 involvement in diabetic complications.

Metformin is a first-line drug in the treatment of Type 2 Diabetes Mellitus. It demonstrates suppressive effects on the NLRP3 inflammasome by inhibiting caspase-1 and the production of IL-1β.(103) Apart from lowering insulin resistance, it has

been shown that Metformin has protective properties against cell pyroptosis and myocardial ischemia–reperfusion injury by interfering with the AMPK/TOR signaling pathway.(8)

Dapagliflozin is a versatile drug that belongs to the SGLT-2 inhibitors. It exhibits both anti-diabetic and cardioprotective features by significantly decreasing NRLP3 activation. In murine models, it has been shown to improve left ventricular end-systolic and end-diastolic volumes as well as the left ventricular ejection fraction by modulating the AMPK/TOR pathway.(155) Empagliflozin, a different SGLT-2 inhibitor, has been tested in experimental models of heart failure without DM. It mitigated the clinical manifestation of heart failure by suppressing the NLRP3 inflammasome and lowering intracellular Ca^{2+}.(156)

Saxagliptin is a Dipeptidyl peptidase-4 inhibitor (DPP4 inhibitor) that has been shown to mitigate the advancement of diabetic cardiomyopathy primarily by restricting NRLP3 inflammasome activation.(8)

Pioglitazone is an anti-diabetic drug that belongs to thiazolidinediones. It demonstrates its inhibitory effect on NLRP3 by downregulating NF-κB. Pioglitazone reduces ROS releases and has been shown to attenuate renal damage.(157)

Acarbose is an a-glucosidase inhibitor that enhanced endothelial function in the aorta of diabetic rats. It suppressed NLRP3 activation by inhibiting NOX4-depedant superoxide production. (158)

5.1.3 Other pharmaceutical options

Eplerenone is an aldosterone antagonist type of potassium-sparing diuretic that is used to treat chronic heart failure and hypertension. In murine models, eplerenone exhibited robust anti-inflammatory properties by blocking transcription of the NLRP3 inflammasome's components, phosphorylation of NF-κB and ROS production.(159)

Verapamil is a non-dihydropyridine calcium channel blocker approved for treating arterial hypertension and angina pectoris. Verapamil when tested in diabetic mice, inhibited the NRLRP3 inflammasome and attenuated pathological neo-angiogenesis.(8)

Fenofibrate, a PPARa agonist, is a drug used for hypertriglyceridemia with additional potential therapeutic benefits in diabetic retinopathy. In animal experiments led to attenuation of retinal leukostasis, vascular leakage and the progression of DR. Although these effects are not officially acknowledged, the NLRP3 inhibition seems to be the basic underlying mechanism.(160)

β-hydroxybutyrate, a ketone body that acts as an alternative ATP source during energy shortages, has been demonstrated to counteract the activating effects of ATP, monosodium urate, and ceramide on the NLRP3 inflammasome by reducing K^+ efflux and inhibiting ASC oligomerization. Moreover, in a mouse model, a ketogenic diet significantly inhibits caspase-1 activation and reduces neutrophil count and hyperglycemia. However, the impact of β-hydroxybutyrate on the adipose tissue inflammasome is yet to be explored.(161)

Statins are widely used lipid-lowering drugs. A plethora of clinical studies suggest that statins, apart from cholesterol reduction, have anticoagulant, anti-inflammatory

and immunomodulatory properties. Wu et al. showed that atorvastatin inhibited the expression of NLRP3 and pyroptosis related molecules. Additionally, in murine models of diabetic cardiomyopathy, atorvastatin ameliorated diastolic dysfunction and cardiac fibrosis. The effects were dependent on the inhibition of NLRP3 inflammasome via TXNIP.(8,162) Simvastatin, exhibits atheroprotective actions via modulating transcription molecules to subsequently suppress the NRLP3 inflammasome.(20)

5.1.4 Natural Substances and Derivatives as NRLP3 inhibitors

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a highly concentrated polyphenol abundant in red grapes, soybeans, peanuts and mulberries. Resveratrol demonstrates antioxidant, anti-inflammatory, and antiaging effects and shows similar properties to metformin in modulating AMPK signaling pathway.(128,163) Resveratrol can mitigate ER stress and mitochondrial fission in adipose tissue by decreasing IRE1α and eIF2α phosphorylation in an AMPK-dependent manner, ultimately suppressing NLRP3 inflammasome activity. Oral supplementation of resveratrol in diabetic mice led to adequate restriction of inflammation and adipose dysfunction.(164)

Berberine is a natural alkaloid found in many medicinal herbs with inhibitory effect on NRLP3. Berberine enhances AMPK-dependent autophagy and eliminates ROS. Oral administration of berberine in HFD-fed murine models improved insulin sensitivity and glucose tolerance.(165)

Parthenolide is an alkylating herbal agent with inhibitory activity towards NRLP3 inflammasome. It disrupts the ATPase activity of NLRP3 via NACHT alkylation.

However, parthenolide is non-selective as it also inhibits NRLP1 ,NLRC4 and caspase-1 and thus may demonstrate off-target effects when used as NLRP3 inhibitor.(24,128)

Melatonin, a hormone produced by the pineal gland that regulates circadian rhythm, suppresses NF-κB signaling by decreasing NF-κB and p65 protein levels in the cytoplasm and nucleus, respectively.(128) In experimental HFD-fed murine models, melatonin injections led to profoundly suppressed protein expression of NRLP3 and serum levels of IL-1β. Furthermore, melatonin treatment marked a profound decrease in adipose tissue pyroptosis through downregulation of caspase-1, GSDMD and interferon regulatory factor 7 (IRF7).(166)

Glycyrrhizin (GL) and Isoliquiritigenin (ILG), the bioactive components of the Glycyrrhiza plant, have been associated with the inhibition of TLR4 signaling. This suppression subsequently decreases downstream NF-κB and mitogen-activated protein kinase (MAPK) activation, ultimately downregulating NLRP3 transcription.(128) Furthermore, their suppressive effects extend beyond the priming phase, as both GL and ILG reduce ASC oligomerization in response to ATP, thereby weakening the activation signal of the NLRP3 inflammasome. In mice models, treatment with Isoliquiritigenin diminishes Il-1β production and adipose tissue inflammation.(167)

5.1.5 Inhibition of Caspase-1, IL-1 and IL-18

Inhibiting inflammasome components such as ASC and caspase-1 or targeting inflammasome-derived cytokines like IL-1β and IL-18, can result in effects similar to NLRP3 inhibition. However, since these elements are not exclusive to the NLRP3 inflammasome, their suppression may also disrupt the function of other inflammasomes.(9)

Not much data is available regarding ASC inhibitors as the few that have been developed were not tested in models of cardiovascular disease.

Caspase-1 inhibition tested both in vitro and ex vivo models did show enhancement of heart contractility and mitigation of cardiomyocyte damage.(168,169)

Contrary to ASC inhibitors, numerous IL-1 inhibitors have been put to test in clinical scenarios throughout the years, although not a single one has been authorized as a treatment option in cardiovascular diseases. IL-1 has well-known destabilizing effects in the atherosclerotic burden of the vessels. As a matter of fact, , deletion of the IL-1RI receptor or IL-1α and IL-1β in murine models diminished the size of the plaques.(109) IL-1a has crucial impact on the very first stages of experimental atherogenesis while IL-1β is associated with plaque remodeling in the late phases of atherosclerosis.(101) The critical role of Il-1β in atherosclerotic disease was proven in the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). In this study, Canakinumab, a humanized monoclonal antibody that neutralizes IL-1β and not IL-1a, significantly reduced the frequency of atherothrombotic events. It should be highlighted that the CANTOS trial is the most extensive study on cytokine inhibition conducted to date, offering several thousand patient-years of

exposure.(26,170) Diving in to more detail, the CANTOS trial stands as a pioneering investigation involving 10,061 individuals who had previously experienced a myocardial infarction and exhibited elevated high-sensitivity C-reactive protein (hs-CRP) levels above 2 mg/L. Participants were assigned to receive subcutaneous injections every three months of canakinumab at doses of 50 mg, 150 mg, or 300 mg, or a placebo. The trial's primary goal was to assess a composite outcome consisting of nonfatal heart attacks, nonfatal strokes, and deaths due to cardiovascular causes. A key secondary endpoint expanded on this by including urgent revascularization procedures, an especially relevant measure when evaluating therapies aimed at halting the advance of atherosclerosis. What sets the CANTOS trial apart is not just its rigorous methodology but its audacious leap from cutting-edge molecular science to clinical application. It served as a vital real-world test of whether blocking interleukin-1β (IL-1β), a central inflammatory mediator, could offer protection in patients with stable coronary artery disease. This landmark research offered definitive proof that the IL-1 signaling pathway plays a significant role in destabilizing atherosclerotic plaques. Moreover, the findings underscore the promise of IL-1 inhibition as a strategy to favorably alter the prognosis for patients already grappling with coronary atherosclerosis.(171–173)

Anakinra, a recombinant form of the human IL-1Ra , is a chimeric protein that suppresses IL-1a and IL-1β. In a murine experimental model of acute myocardial infarction due to permanent coronary artery occlusion, Anakinra lessened the size of the infarcted area, restricted the adverse ventricular remodeling and improved the ejection fraction of the left ventricle of the murine heart.(9) The Virginia Commonwealth University Anakinra Remodeling Trials (VCUART) was a three consecutive double-blinded placebo-controlled clinical studies that evaluated patients

with ST segment elevation Myocardial Infarction (STEMI). In comparison to the placebo group, patients treated with Anakinra had lower levels of CRP. Additionally, patients in the Anakinra group had notably lower incidence of heart failure onset.(170,174,175) In the Recently Decompensated Heart Failure Anakinra Response Trial (REDHART), anakinra enhanced cardiorespiratory fitness (peak oxygen consumption), lowered NT-proBNP levels, and boosted the patients' quality of life.(176)

IL-18 has equally significant effects on the cardiovascular system. Augmented IL-18 serum levels were negative predictors of acute myocardial events. Furthermore, increased levels of IL-18 were associated with great severity of heart failure.(9) In murine ischemia-reperfusion models, an antibody neutralizing IL-18 prior to injury led to smaller infarct size.(177) Additionally, treatment with a recombinant IL-18 binding protein (IL-18BP) reduced heart damage and inflammation in a mouse model of heterotopic heart transplantation.(178) In an in vitro ischemia model, human myocardial strips treated with IL-18BP demonstrated improved contractility compared to the controls.(9) Lastly, administering an antibody against IL-18 to mice with myocardial injury induced by β-adrenergic receptor overstimulation resulted in reduced heart damage, decreased fibrosis, and enhanced myocardial function.(179)

5.2 Genetic approach of NRLP3 inhibition

Aside from pharmacological methods, the direct genetic deletion of NLRP3 remains primarily limited to molecular research and is rarely utilized in clinical practice, mainly due to safety concerns. Encouragingly, CRISPR/Cas9, a third-

generation gene-editing technology, was first employed to target NLRP3 in peritoneal macrophages using an in vivo delivery system consisting of cationic lipid-assisted nanoparticles encapsulating mCas9 and gRNA. This approach has proven effective in counteracting various inflammatory diseases, as demonstrated by the alleviation of HFD-induced type 2 diabetes and LPS-induced septic shock in NLRP3 knockout mice. However, additional research is necessary to evaluate potential immune-related side effects, given the essential role of the NLRP3 inflammasome in innate immunity.(128,180)

6. Discussion

Inflammation is a crucial factor in the onset and progression of T2DM and its associated macrovascular complications. The clinical and experimental findings presented in this review emphasize on the significance of systemic and localized low-grade chronic inflammation as a fundamental driving force in the development of metabolic disorders like T2DM. Recent randomized controlled trials have validated the advantage of directly addressing inflammation in cardiovascular disease, particularly in the context of T2DM.(110,181)

In recent years, the involvement of the NLRP3 inflammasome in the initiation and progression of diabetes has become increasingly evident. As a crucial regulator of immune and inflammatory responses, it plays a significant role in the development of T2DM. The upregulation of NLRP3 has been linked to nearly all major diabetes-related complications affecting multiple organ systems, especially the cardiovascular system.(8) The NLRP3 inflammasome contributes to diet-induced insulin resistance, immune dysfunction, plaque formation, and vascular remodeling. Additionally, it

amplifies various age-related metabolic disturbances in adipose tissue, including impaired glycemic control, increased visceral fat accumulation, and reduced lipolysis. Studies across different human populations have identified a positive correlation between NLRP3-driven inflammation in adipose tissue and cardiometabolic disorders, though a direct causal link has yet to be established.(182)

Given the critical role of the NLRP3 inflammasome in the human body, its suppression could offer a novel strategy for mitigating hyperglycemic toxicity and preventing the development of vascular complications in individuals with T2DM.(21) Although a variety of anti-diabetic, lipid-lowering, anti-hypertensive and plenty other medications have demonstrated inhibitory potential on NLRP3, their effects on NLRP3 modulation are off-label and are yet to be approved for clinical use. Current efforts in treating NLRP3-related cardiovascular diseases have focused on the development of drugs targeting IL-1β, such as Anakira and Canakinumab, direct NRLP3 inhibitors and the usage of natural substances that seem have inhibitory properties on NLRP3. Among direct NLRP3 inhibitors (MCC950, tranilast, OLT1177, INF39) tranilast is the most extensively studied in humans. However, no pharmacological treatments have been authorized for clinical use by medical institutions.(8) Despite significant advancements, our knowledge of inflammasome biology remains incomplete, limiting its full potential for developing anti-inflammatory therapies. Gaining deeper insights into the activation mechanisms, assembly processes, and upstream signaling pathways of various inflammasomes, both at the transcriptional and post-transcriptional levels, is crucial for identifying new therapeutic targets.(12) In summary, rigorously controlled experimental studies are essential to identify and evaluate novel agents that specifically target

inflammasomes in diabetic complications in order to advance to a growing number of human clinical trials.(2)

7. Conclusion

This review explored the complex role of the NLRP3 inflammasome in the pathogenesis of Type 2 Diabetes Mellitus (T2DM) and its macrovascular complications, highlighting both its molecular mechanisms and therapeutic potential.

The main findings are as follows:

- Chronic low-grade inflammation is a hallmark of T2DM, with IL-1β and IL-18, products of NLRP3 activation, playing a central role in β-cell dysfunction, insulin resistance, and systemic metabolic imbalance.
- The NLRP3 inflammasome is activated through various mechanisms, including K^+ efflux, ROS production, lysosomal rupture, and mitochondrial dysfunction. These are common in obesity, hyperglycemia, and nutrient overload—all key features of T2DM.
- NLRP3 activation contributes to endothelial dysfunction, a precursor to atherosclerosis, myocardial infarction, stroke, and peripheral artery disease in diabetic patients.
- Studies in both animal models and human tissues have shown that NLRP3 expression is elevated in adipose tissue, monocytes, and vascular lesions of patients with T2DM and related cardiovascular conditions.

- A range of pharmacological agents, such as MCC950, OLT1177, glyburide derivatives, and colchicine, have shown promising anti-inflammatory and anti-atherosclerotic effects by directly or indirectly inhibiting NLRP3 activation.
- Genetic deletion or inhibition of NLRP3 in experimental models leads to improved glucose tolerance, reduced insulin resistance, and protection against vascular damage.

In summary, the NLRP3 inflammasome represents a critical link between metabolic dysfunction and inflammation, offering a compelling target for future therapies. While preclinical data are encouraging, further clinical trials are essential to determine the efficacy, safety, and long-term outcomes of NLRP3-targeted treatments in humans. Ultimately, integrating inflammasome inhibition into diabetes care may help curb the burden of cardiovascular disease and improve quality of life in T2DM patients.

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9. Appendix

Figure 1. NLRP3 inflammasome inhibitors tested in the cardiovascular system and their site of action. MCC950 and CY09 directly bind to the NACHT domain, OLT1177 and INF4E impair ATPase activity of NLRP3, Colchicine inhibits the spatial arrangement of NLRP3 and ASC, Tranilast blocks the direct NLRP3-NLRP3 and NLRP3–ASC interaction, Hydrogen Sulfide reduces NLRP3-dependent caspase-1 activation

(NLRP3; NACHT, leucine-rich repeat (LRR) and pyrin domain (PYD)-containing protein 3; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD), GSDMD; Gasdermin D, GSDMS-N; GSDMDN-terminal fragment, proIL-1β; pro interleukin 1-β, proIL18; pro interleukin 18, IL-1β; interleukin 1-β, IL-18; interleukin18)

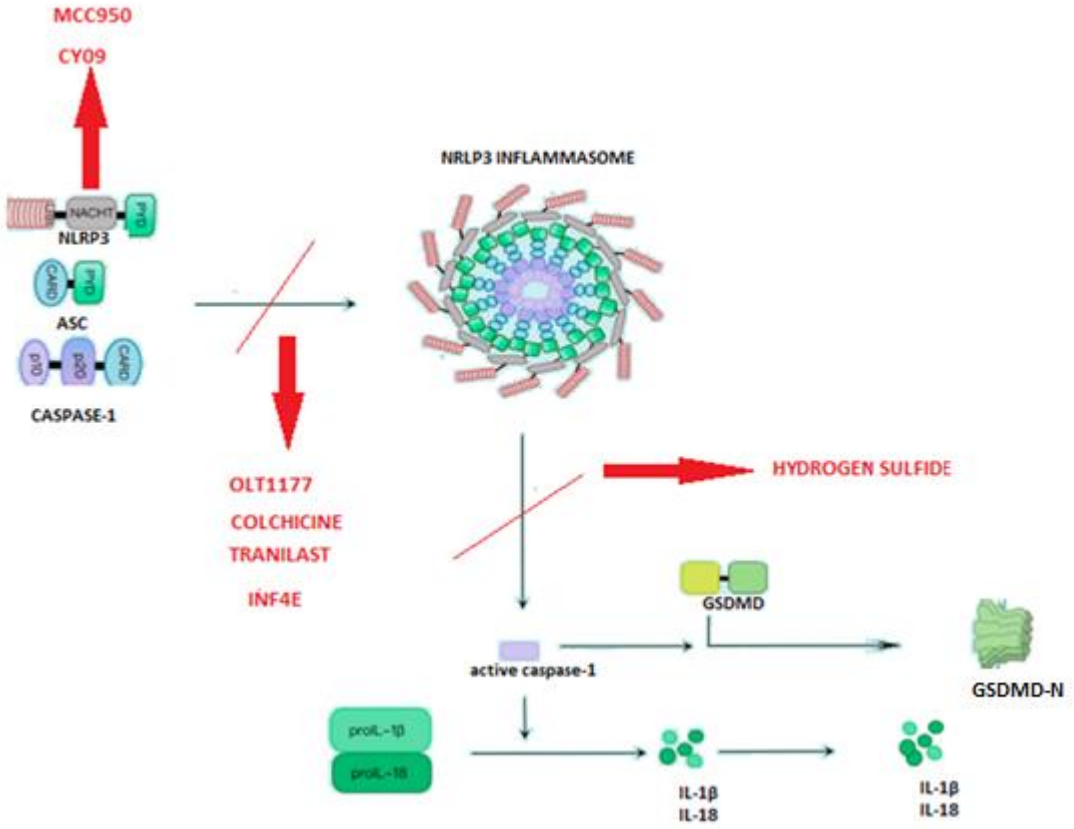

Table 1. Summary of studies indicating correlation between NRLP3 and Type 2 Diabetes Mellitus (T2DM)				
Authors	Year	Population	Study	Findings
Esser et al.(83)	2013	Human participants with different obesity phenotypes	Cross-sectional observational study	Increased expression of NLRP3 and IL1B in visceral adipose tissue from metabolically unhealthy obese patients
Yin et al. (84)	2014	Postmenopausal women, both lean and obese, undergoing elective abdominal surgery	Cross-sectional observational study	Genes associated with the NOD-like receptor pathway, including the NLRP3were upregulated in adipocytes from obese individuals
Wang et al.(85)	2015	db/db mice	Pre-clinical experimental study (with in vivo and vitro methodologies)	NLRP3 and Caspase-1 expressions were increased in epididymal fat from db/db mice
Finucane et al.(86)	2015	C57BL/6 mice	Pre-clinical experimental study	NLRP3, Caspase-1, and IL1B expressions in adipose tissue were higher in mice treated for 6 months with a saturated fatty acid HFD in comparison with mice fed with a monounsaturated fatty acid HFD
Bitto et al.(87)	2014	db/db mice	Pre-clinical experimental study	NLRP3, ASC, caspase-1, IL-18, and IL-1 are upregulated during wound healing in animal models of T2DM in comparison with healthy animals
Coll et al.(88)	2019	Mouse bone marrow-derived macrophages and human monocyte-derived macrophages	Pre-clinical experimental study	MCC950, which inhibits the NLRP3 inflammasome, can be applied as a potential anti-

				inflammatory therapy in T2DM
Henriksbo et al.(89)	2014	ob/ob mice, 3T3-L1 adipocytes (murine adipocyte cell line)	Pre-clinical experimental study (with in vivo and vitro methodologies)	Fluvastatin provokes inflammation and insulin resistance in adipose tissue via the upregulation of NLRP3, which is consistent with the increased expression of NLRP3 in inflamed adipose tissues of T2DM patients
Kim et al.(90)	2016	Murine macrophage cell lines(iJ774) and bone marrow-derived macrophages(BMDMs)	Pre-clinical experimental study (with in vivo and vitro methodologies)	NLRP3 can be suppressed by γ-tocotrienol, delaying the progression ofT2DM

Table 2. Summary of studies correlating NRLP3 inflammasome with Macrovascular disease in Type 2 Diabetes Mellitus(T2DM)				
Authors	Year	Population	Study	Findings
Ridker et al. CANTOS trial (101)	2017	Patients with history of myocardial infarction and elevated hsCRP levels	Randomized, double-blind, placebo controlled, multicenter clinical trial	150mg of Canakinumab significantly reduced cardiovascular death, providing the first definitive clinical evidence that reducing inflammation can lower CVD event risk
Yin Jin et al.(102)	2022	ApoE-/- mice	Pre-clinical experimental study	Targeting caspase-1 and the NLRP3 assembly may offer therapeutic potential in atherosclerotic cardiovascular diseases.
Lee et al. (103)	2013	Patients with untreated T2DM	Comparative experimental study	Increased expression of the inflammasome components NLRP3 and ASC was found in monocytes from newly identified, untreated type 2 DM subjects
Luo et al. (104)	2014	HFD- and STZ-induced rat models	Pre-clinical experimental study	Diabetic rats showed severe metabolic disorder, cardiac inflammation, cell death, disorganized ultrastructure, fibrosis and excessive activation of NLRP3
Wan et al. (105)	2019	Humans and ApoE−/− mice	Pre-clinical experimental study (with in vivo and vitro methodologies)	NLRP3 was involved in hyperglycemia-induced endothelial inflammation, both in vitro and in vivo
Xiao-Xue Li et al. (106)	2019	Diabetic rats	Pre-clinical experimental study	High glucose induced the assembly and activation of NLRP3 inflammasome in endothelial cells
Feng et al. (96)	2016	Rat glomerular mesangial cells	Pre-clinical experimental study	High glucose levels and LPS exposure prime the NRLP3 inflammasome in mesangial cells through ROS/TXNIP signaling

				pathway leading to Diabetic Nephropathy
Sun et al. (107)	2019	STZ-induced diabetic rat model	Pre-clinical experimental study	Suppression of TXNIP/NLRP3 activation ameliorates Diabetic Peripheral Neuropathy
Yu Li et al. (108)	2013	Porcine model of atherosclerosis and DM	Pre-clinical experimental, in vivo, study	In vivo evidence that the dysregulation of SIRT1-AMPK-SREBP and stimulation of NLRP3 inflammasome may contribute to vascular lipid deposition and inflammation in atherosclerosis.
Duewell et al. (109)	2010	Mice deficient in components of the NLRP3 inflammasome	Pre-clinical experimental study (with in vivo and vitro methodologies)	Crystalline cholesterol acts as an endogenous danger signal and its deposition in arteries or elsewhere is an early cause rather than a late consequence of NLRP3 activation and inflammation.
Kirii et al. (110)	2003	apoE−/− and IL-1β−/−mice	Pre-clinical experimental, in vivo, study	IL-1β deficiency significantly reduced atherosclerotic lesion size in the aorta suggesting that IL-1β promotes atherogenesis through both immune cell recruitment and endothelial activation.
Qian An et al. (111)	2017	STZ-induced diabetic rats	Pre-clinical experimental, in vivo, study	Suppression of the NLRP3 inflammasome pathway via oleanolic acid attenuates carotid artery injury in diabetic rats
Song et al. (112)	2015	Cultured endothelial cells	Experimental, in vitro, cellular study	Inhibition of ER stress-associated TXNIP/NLRP3 inflammasome activation in endothelial cells improves endothelial homeostasis.

Table 3. Pharmacological approaches of NLRP3 inhibition

	Drugs	Mechanism of action	Studies	Findings	Status
NLRP3 inhibitors	MCC950 (73,129,130)	Non-covalent bonding to the NACHT domain	Many murine models(HFD, streptozotocin-induced ApoE$^{-/-}$ mice etc.) and Humans	Reduced atherosclerotic plaque development, decreased the expression of adhesion molecules within the plaque, and lowered the number of macrophages present in the plaque	Clinical development was discontinued due to excessive renal inflammation and hepatic toxicity
	Glyburide (135–137)	Inhibition of ATP-dependent potassium channels	Murine and Humans models	Suppressed cardiac caspase-1 activity and minimized infarct size in mice undergoing myocardial ischemia followed by 24 hours of reperfusion	Limited clinical use due to frequent hypoglycemia
	Bay 11-7082 (139,140)	NF-κB pathway inhibition	Myocardial ischemia-reperfusion murine models	Decreases leukocyte infiltration in the infarcted area and enhances cardiomyocyte survival, reducing infarct size	Pre-clinical studies
	OLT1177 (141–143)	Impairs ATPase activity of NLRP3	Animal models of myocardial ischemia-reperfusion	Dose-dependent reduction in infarct size, also improved ventricular	Pre-clinical studies

				function in a model of permanent coronary artery occlusion	
	Colchicine (144–147)	Interferes with the NLRP3 complex by disrupting microtubule action	Human studies (COLCOT, LoDoCo) and Mouse models of permanent cardiac ligation	Decreased the infiltration of neutrophils and macrophages, as well as the mRNA expression of pro-inflammatory cytokines and NLRP3 inflammasome components 24 hours after myocardial infarction	FDA approved for inflammatory diseases
	CY-09 (13,149)	Inhibition of the NLRP3 complex by binding directly to the ATP-binding motif of the NACHT domain	Murine models of type 2 Diabetes Mellitus	Prevented cardiac dysfunction linked to diabetic ischemic stroke	Pre-clinical studies
	Tranilast (9,137,150)	Blocks the direct NLRP3-NLRP3 and NLRP3–ASC interaction	Mouse models of atherosclerosis and several animal models of hypertension, diabetic cardiomyopathy and myocardial infarction	Enhanced NLRP3 ubiquitination, restricting NLRP3 inflammasome assembly and thereby reducing the initiation and progression of atherosclerotic plaques	Pre-clinical studies
	INF4E (151,152)	Inhibition of the NLRP3 ATPase activity	Murine models of myocardial ischemia	Reduced infarct size and improved left ventricular pressure	Clinical development was discontinued due to cytotoxic properties

	Hydrogen Sulfide (16,153,154)	Reduces NLRP3-dependent caspase-1 activation	Murine specimen undergoing ischemia-reperfusion injury	Diminished the IKKβ/NF-κB signaling pathway introducing cardioprotective properties in a hemorrhagic shock model	Pre-clinical studies
Anti-Diabetic Drugs	Metformin (8,103)	Activates AMPK that reduces ER stress and mitochondrial fission leading to inhibition of caspase-1	Studies in Monocyte-derived macrophages isolated from type 2 diabetic subjects	Protective properties against cell pyroptosis and myocardial ischemia–reperfusion injury by interfering with the AMPK/TOR signaling pathway	FDA approved for Type-2 Diabetes Mellitus
	SGLT2 inhibitors (155,156)	Modulatory effects on the AMPK/TOR pathway	Eight-week-old BTBR and wild-type mice	Improved left ventricular end-systolic and end-diastolic volumes as well as the left ventricular ejection fraction by modulating the AMPK/TOR pathway	FDA approved for Type-2 Diabetes Mellitus and Heart Failure
	Pioglitazone (157)	Downregulation of NF-κB	apoE (-/-) mice	Reduced ROS releases and attenuated renal damage	FDA approved for Type-2 Diabetes Mellitus
	Acarbose (158)	Inhibition of NOX4-depedant superoxide production	Rats with T2D	Enhanced endothelial function in the aorta of diabetic rats	FDA approved for Type-2 Diabetes Mellitus
	Saxagliptin (8)	AMPK-dependent caspase-1 inhibition	Type 2 diabetic (BTBR ob/ob) and wild-type (WT) mice	Mitigate the advancement of diabetic cardiomyopathy	FDA approved for Type-2 Diabetes Mellitus

Other pharmaceutical options	Eplerenone (159)	Inhibits phosphorylation of NF-κB and ROS production	C57BL/6 mice fed a high-fat diet (HFD)	Exhibited robust anti-inflammatory properties	FDA approved drug for Hypertension and Heart Failure
	Verapamil (8)	Inhibits the assembly of NLRP3, reduces the release of IL-1β	Mouse models with Diabetic Retinopathy	Attenuated pathological neo-angiogenesis	FDA approved drug for Hypertension and Angina Pectoris
	Fenofibrate (160)	Unidentified mechanism of NRLP3 inhibition	Mouse models with Diabetic Retinopathy	Attenuated retinal leukostasis, vascular leakage and the progression of DR	FDA approved for hypertriglyceridemia
	Atorvastatin (8,162)	Inhibition of NLRP3 inflammasome via TXNIP	Murine models of diabetic cardiomyopathy	Ameliorated diastolic dysfunction and cardiac fibrosis	FDA approved lipid lowering agent
	β-hydroxybutyrate (161)	Abolishes K+ efflux; Reduces ASC oligomerization and speck formation via unknown mechanism	Mouse models of ketogenic diet	Inhibited caspase-1 activation, reduced neutrophil count and hyperglycemia	Pre-clinical studies
****** All above-mentioned FDA approved drugs have **off-label effects** in NRLP3 inhibition and their underlying mechanisms are still in **pre-clinical research** for future development of related compounds******					
	Resveratrol (128,163,164)	Modulation of AMPK signaling pathway	Diabetic murine models	Restriction of inflammation and adipose dysfunction	
	Berberine (165)	Enhances AMPK-dependent autophagy	HFD-fed murine models	Improved insulin sensitivity and glucose tolerance	

Natural Substances	Parthenolide (24,128)	Impairs ATPase activity of NLRP3; Suppresses IκB kinase and NF-κB	mouse ASC (polyclonal anti-mouse ASC), mouse NLRP3 (polyclonal anti-NLRP3 PYD), mouse caspase-1 p20 (monoclonal anti-mouse caspase-1 p20)	Exhibited anti-inflammatory properties via macrophage blockage	Pre-clinical studies
	Melatonin (166)	suppresses NF-κB signaling by decreasing NF-κB and p65 protein levels in the cytoplasm and nucleus	HFD-fed murine models	Profound decrease in adipose tissue pyroptosis	
	Glycyrrhizin (GL) and Isoliquiritigenin (ILG) (128,167)	Inhibits mitogen-activated protein kinase (MAPK) activation	HFD-fed murine models	Diminished Il-1β production and adipose tissue inflammation	