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Article

NLRP3 inflammasome in metabolic syndrome

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Metabolic syndrome (MS) is a serious health problem worldwide; it is characterized by a group of metabolic disorders, including central obesity, insulin resistance/type 2 diabetes, hyperlipidemia with accelerated atherosclerosis, hypertension, non-alcoholic fatty liver disease, and elevated uric acid with increased risk of gout. The incidence of MS has increased considerably in recent decades and has attracted considerable attention. A number of clinical and translational laboratory studies have implicated the activation of nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome in the development of MS, therefore establishing a strong link between chronic inflammation and metabolic diseases. This paper aims to review new developments on NLRP3 inflammasome in MS for better understanding of chronic inflammation in metabolic diseases. We will also provide new insights into using NLRP3 inflammasome as an innovative therapeutic target.

Keywords: Metabolic syndrome. NLRP3 inflammasome. Chronic inflammation. IL-1β

INTRODUCTION

Accumulating evidence strongly links chronic inflammation to metabolic syndrome (MS). A number of recent landmark studies have demonstrated that chronic inflammation is the key feature and basis of MS (Hotamisligil, 2006; Fève and Bastard, 2009). A wide variety of immune cells, such as macrophages, monocytes, and T cells, have been shown to infiltrate the adipose tissue, liver, and pancreatic islets in the development of MS (Stienstra *et al*., 2012). Additionally, numerous pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukins (ILs), and adipokines which are secreted by adipocytes, participate in the pathogenesis of MS. More recently, interest has been focused on the role of a multiprotein complex called nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome, which controls the processing and production of IL-1β and IL-18. Structurally, the NLRP3 inflammasome consists of three components: NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and procaspase-1. The NLRP3 inflammasome is assembled in response to diverse exogenous and endogenous danger signals. Recent findings reveal that NLRP3 inflammasome can be activated by metabolic danger signals, such as extracellular ATP, glucose, islet amyloid polypeptide (IAPP), free fatty acids, oxidized low-density lipoprotein (LDL), cholesterol crystals, and uric acid (Mori, Bezy and Kahn, 2011). The activation of NLRP3 inflammasome then leads to autocatalytic activation of caspase-1 and subsequently cleaves inactive pro-IL-1β and pro-IL-18 into their bioactive forms: mature IL-1β and IL-18, respectively. IL-1β is a prominent pro-inflammatory cytokine that can efficiently elicit potent pro-inflammatory actions by binding to the IL-1 receptor. This IL can also cause the generation of other inflammatory mediators, including TNF-α and IL-6, thus initiating a self-amplifying cytokine network that promotes MS progression (Arend, Palmer and Gabay, 2008).

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NLRP3 INFLAMMASOME IN OBESITY

To date, approximately 30% of the adult population suffers from obesity. Alarmingly, according to the World Health Organization, in 2016, more than 1.9 billion adults (39%) were overweight, and 650 million of them were obese (13%) (Racz *et al*., 2018).

Obesity is suggested to be a chronic low-degree inflammatory state with pro-inflammatory mediators, such as TNF-α, IL-1β, IL-6, and leptin, infiltrating adipose tissues. Thus, obesity could also be called "obesitis" (Duncan and Schmidt, 2001). Recently, a number of studies have shed light on the pathogenesis of obesity and indicated that NLRP3 inflammasome is an important contributor to such process (Esser *et al*., 2013), (Benetti *et al*., 2013). During the progression of obesity, adipose tissue macrophages are activated within fat deposits, and NLRP3 inflammasome can sense the sterile danger signals originating from fat tissues. Obesity features elevated circulating levels of free fatty acids, especially palmitate which is one of the most abundant saturated fatty acids in plasma. Interestingly, studies have recently reported that palmitate can activate NLRP3 inflammasome (Wen *et al*., 2011). In macrophages, palmitate induces the activation of caspase-1 and cleavage of IL-1β in a NLRP3-dependent manner. Detailed mechanistic studies showed that palmitate reduces the activation of AMP-protein kinase (AMPK), which is an energy-sensing kinase that regulates lipid and glucose metabolism. The reduction of AMPK activity leads to defective autophagic process and subsequent accumulation of mitochondrial-derived reactive oxygen species (ROS). Previous findings have proposed that ROS are a requirement for NLRP3 inflammasome activation, consistent with the results of this study (Tschopp and Schroder, 2010), (Zhou *et al*., 2011). In addition, ceramide is another endogenous danger signal originating with obesity; it specifically results from the metabolism of long-chain saturated fatty acids. *Lipopolysaccharide* (LPS)-primed macrophages stimulated with ceramide display NLRP3 inflammasome activation. Likewise, ceramide causes NLRP3 activation and the release of IL-1β in macrophages in wild-type mice but not in $NLRP^{-/-}$ mice (Vandanmagsar *et al*., 2011). In comparison with calorie-restricted diet-fed mice, normal diet-fed mice display increased expression of NLRP3 and IL-1β in visceral adipose tissue, and this finding has been found to correlate with body weight directly. Strikingly, clinical studies revealed that calorie restriction and weight loss can reduce NLRP3 expression in abdominal subcutaneous adipose tissue in obese patients, accompanied by decreased inflammation (Vandanmagsar *et al*., 2011). Thus, other unknown endogenous signals might trigger NLRP3 inflammasome activation in obesity, which is a primary cause of insulin resistance and type 2 diabetes (T2D).

NLRP3 Inflammasome in Insulin Resistance and T2D

T2D is characterized by obesity-induced insulin resistance and dysfunction of islet β cells in the pancreas. Notably, NLRP3 inflammasome activation in adipocytes was recently reported to impair insulin sensitivity (Stienstra *et al*., 2010). In line with this condition, adipocytes isolated from NLRP3-deficient mice showed a significantly reduced production of IL-1β and increased insulin sensitivity. Furthermore, ablation of NLRP3 in mice has also been reported to improve insulin sensitivity and glucose homeostasis (Vandanmagsar *et al*., 2011). To confirm the clinical relevance of the data generated from mouse models, the same authors have demonstrated that NLRP3 inflammasome is associated with insulin resistance in obese humans with T2D. The direct effect of NLRP3 inflammasome activation involves the maturation and release of IL-1β, which promotes β cell dysfunction and cell death directly. Additionally, IL-1β impairs insulin signaling in insulin targets, such as liver, muscle, and adipose tissue. Another hallmark feature of T2D is pancreatic deposition of IAPP, which is secreted together with insulin by the β cell, leading to loss of β cell mass (Quan, Jo and Lee, 2013). A recent study showed that IAPP can induce macrophages to produce IL-1β in a NLRP3-dependent manner (Masters *et al*., 2010). When a human IAPP transgene is overexpressed in mouse, which is unable to form active amyloid aggregates, amyloid accumulates in pancreatic islets and energizes NLRP3 inflammasome, subsequently promoting caspase-1 cleavage and IL-1β production. Mechanistically, instigation of NLRP3 inflammasome by IAPP might involve ROS production as ROS inhibitors protects β cells from IAPP-mediated apoptosis (Zraika *et al*., 2009). Remarkably, IL-1β antagonists effectively improve glycemic control and β cell mass in patients with T2D, showing that IL-1β is a key player in this disease (Malozowski and Sahlroot, 2007). Moreover, specific caspase-1 inhibitors are suggested to feature potential in improving insulin resistance in animal models with

T2D (Lee and Lee, 2014). Interestingly, a widely used sulfonylurea drug, glyburide, has been shown to inhibit NLRP3 inflammasome-mediated caspase-1 activation and the release of IL-1β, which may be part of its effect on treating T2D (Lamkanfi *et al*., 2009). Altogether, these studies have provided evidence supporting that NLRP3 inflammasome plays a critical role in insulin resistance and contributes to T2D progression.

NLRP3 Inflammasome in Atherosclerosis and Cardiovascular Disease (CVD)

Chronic inflammation is well-established as a major driving force in atherogenesis. The pathognomonic feature of atherosclerosis involves the deposition of cholesterol crystal, macrophage recruitment, and infiltration of sites of atherosclerotic plaque by inflammatory mediators. Among inflammatory mediators, IL-1β is a potent pro-atherogenic cytokine both *in vitro* and *in vivo* studies. Deficiency of IL-1β in atherosclerosis-prone ApoE$^{-/-}$ mice leads to attenuated development of atherosclerosis (Kirii *et al*., 2003). In line with this finding, treatment with an antagonist of IL-1 receptor prevents the development of atherosclerotic lesions in the arterial wall, indicating that IL-1β plays an important role in promoting atherosclerosis (Elhage *et al*., 1998). Cholesterol crystals formed in atherosclerotic lesions can induce IL-1β secretion in NLRP3 inflammasome-mediated pathway. However, knocking down NLRP3 in human macrophages completely abolishes cholesterol crystal-induced IL-1β secretion (Rajamäki *et al*., 2010). Consistent with *in vitro* studies, atherosclerotic-prone LDL-receptor-deficient mice that were reconstituted with bone marrow from NLRP3$^{-/-}$, ASC$^{-/-}$, or IL-1β$^{-/-}$ mice developed many fewer atherosclerotic plaques and less aortic lesion size than those reconstituted with wild-type bone marrow (Egan, *et al*., 2011). All these findings suggest that NLRP3 inflammasome activation is a key event in atherosclerosis.

Individuals with atherosclerosis arising within large artery walls often progress to CVDs, such as stroke and myocardial infarction. A number of recent findings have indicated a link between inflammasome activation and CVD development. An experimental study described inflammasome activation in cardiac fibroblasts and infiltrating cells in myocardial ischemia/reperfusion (I/R) mouse model (Kawaguchi *et al*., 2011). During the infarction process after acute myocardial I/R injury in mouse, inflammasome was also detected within the infarct zone. However, mice deficient with ASC or caspase-1 which is a component in NLRP3 inflammasome displayed a decline in myocardial infarct size with reduction of infiltrated inflammatory cytokines (Kawaguchi *et al*.,2011). In conclusion, the evidence above suggest that NLRP3 inflammasome plays an important role in the pathogenesis of CVD.

NLRP3 Inflammasome in Non-alcoholic Fatty Liver Disease (NAFLD)

NAFLD is considered one of the manifestations of MS and the leading cause of chronic liver disease in the Western world. The prevalence of NAFLD reaches 30% in the general population and up to 75%–100% in obese individuals (Henao-Mejia *et al*., 2014). A total of 20% of NAFLD individuals are assumed to experience non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis, portal hypertension, and hepatocellular carcinoma (Henao-Mejia *et al*., 2014). Interestingly, the role of NLRP3 inflammasome in NAFLD/NASH progression is receiving increasing attention. Enhanced expressions of inflammasome components were detected in isolated hepatocytes from NAFLD. NLRP3 inflammasome activation was also observed in the liver of humans with NASH. Studies proposed that potential molecular triggers for NLRP3 inflammasome activation in NAFLD/NASH include saturated fatty acids (such as palmitate), LPS, and DNA (Szabo *et al*., 2012). Nevertheless, mice lacking NLRP3, ASC, or caspase-1 have shown different effects on NAFLD progression. ASC$^{-/-}$ mice displayed decreased hepatosteatosis and liver triglyceride levels. However, mice with NLRP3 or caspase-1 knockout failed to show this change. Thus, researchers suggested that the different results were due to alterations in intestinal microbiota communities associated with multiple inflammasome deficiencies (Henao-Mejia *et al*., 2012). The additional distinct mechanisms underlying such condition may need to be elucidated in the following studies.

NLRP3 Inflammasome in Gout

Gout is a sterile inflammatory disease characterized by deposition of monosodium urate (MSU) crystals in joints (Bardin, *et al*., 2014). Gout is also a metabolic disorder that often occurs in obese and T2D patients, as hyperinsulinemia has been suggested to lead to hyperuricemia, which is a main risk factor for gout

development. Uric acid is produced by dying cells as final products of purine degradation, whereas the "danger signal" (MSU crystals) form after the release of uric acid. Interestingly, MSU crystals have been identified as an inducer of NLRP3 inflammasome both *in vitro* and *in vivo* (Martinon *et al*., 2006). Cells from the differentiated monocytic cell line THP-1 stimulated with MSU crystals can release IL-1β in caspase-1-dependent manner, as the caspase-1 specific inhibitor completely blocks MSU-induced IL-1β production, indicating the involvement of NLRP3 inflammasome in this process. Furthermore, macrophages from mice deficient in NLRP3, caspase-1, or ASC (a component of inflammasome), failed to produce IL-1β, demonstrating that MSU crystals can specifically activate the NLRP3 inflammasome. Based on the critical role of NLRP3 inflammasome in MSU recognition, IL-1β blockade by anakinra or rilonacept in gout patients was proven successful (Martinon *et al*., 2006). Collectively, these findings suggest that the NLRP3 inflammasome can sense metabolic-associated danger signal and contribute to gout development.

MS Treatment by Targeting the NLRP3 Inflammasome

Considering that NLRP3 inflammasome is involved in the pathogenesis and development of MS, inhibition of its activation may represent an attractive therapeutic target. IL-1β is the major downstream product of NLRP3 inflammasome activation. To date, the most promising approaches use either IL-1 receptor antagonist or IL-1β neutralizer (Menu *et al*., 2011). A recombinant human IL-1 receptor antagonist anakinra was effective in treating T2D, gout, and pseudogout in clinical trials (So *et al*., 2007). In addition, XOMA 052, which is a high-affinity monoclonal antibody to IL-1β, has shown positive results in mice with atherosclerosis and is suggested as treatment for patients with CVD (Terkeltaub *et al*., 2009). A more preventative therapeutic approach is directly targeting the components of NLRP3 inflammasome. Specifically, caspase-1 inhibitor is beneficial in reducing obesity and has successfully ameliorated insulin resistance and T2D in mice. Such pharmacological agent may be beneficial for humans although no drug is available for human use at the moment. Furthermore, glyburide, which is a widely used drug in treating T2D, has been shown to inhibit NLRP3 inflammasome. Recently, omega-3 fatty acids (w-3FAs) such as eicosapentaenoic acid and docosahexaonoic acid, have been shown to inhibit NLRP3 inflammasome activation and subsequent IL-1β secretion. Importantly, *in vivo*, w-3FAs also prevented high-fat-diet-induced metabolic disorder and T2D through inhibition of NLRP3 inflammasome activation (Jesus, Goldbach-Mansky, 2014; Osborn *et al*., 2008). This finding suggests the potential clinical use of w-3FAs in MS or other NLRP3 inflammasome-driven inflammatory diseases. Given the recent insights into MS treatment by targeting the NLRP3 inflammasome, more therapeutic approaches need to be considered in further studies.

Concluding Remarks and Future Directions

The past decade has made considerable advances in our understanding of how proinflammatory cytokines and specific immune cells promote MS. In particular, the activation of NLRP3 inflammasome, which contributes to pathophysiological mechanisms that explain MS development, is beginning to be characterized in detail. Moreover, substantial progress has been made in discovering potential therapies for targeting NLRP3 inflammasome in combating MS, supported by the results of both preclinical studies and new clinical trials. Despite these advances, a number of unanswered questions remain. One of these questions is whether other NLRP3 stimuli are associated with metabolic stress. Whether other uncharacterized NLRPs functioning as a sensor for metabolic stress beside NLRP3 inflammasome exist also remains unclear. Another open question is how NLRP3 inflammasome facilitates organ crosstalk in different manifestations of MS, thus acting as a crucial signaling pathway. In the following years, studies will focus on revealing the stage at which NLRP3 inflammasome affects MS progression and provide more efficacious approaches at improving metabolic diseases. Further studies will be necessary to clarify these points and allow better understanding of the role of NLRP3 inflammasome in MS diseases, leading to improved therapeutic approaches for their treatment.

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