REVIEW ARTICLE

ROLE OF NLRP3 INFLAMMASOMES IN PATHOGENESIS OF TYPE 2 DIABETES MELLITUS-AN OVERVIEW

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ABSTRACT

Diabetes mellitus type 2 (T2D) is a multifactorial metabolic disorder associated with chronic inflammation. Several mechanisms have been postulated for its pathogenesis. One recently postulated mechanism is based on the activation and assembly of inflammasomes. Intracellular sensors recognize damage associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). This leads to assembly and activation of inflammasomes starting a cascade of events resulting in chronic inflammation in T2D. These inflammasomes are under extensive study to understand the signaling pathways involved in the pro-inflammatory environments among patients of T2D. NLRP3 (Nucleotide binding oligomerization domain, LRRs and pyrins) mediated secretion of cytokines plays a vital role in the pathogenesis of T2D and associated complications. A better understanding of this inflammasome can lead to discovering novel therapies for T2D treatment. Moreover, NLRP3 inhibitors can be used to slow down the progression of disease and to prevent complications in T2D.

Keywords: *Diabetes Mellitus, Damage associated molecular patterns, Pathogen associated molecular patterns, Pyroptosis, NLRP genes*

BACKGROUND

T2D is a major health issue with global impact. It results from interplay of multiple risk factors. Positive family history and environmental factors increase the chances of developing T2D. Chronic inflammatory response remains the backbone of the pathogenesis. Circulating levels of various inflammatory mediators such as interleukins, tumor necrosis factors and adipokines is high especially in obese patients.[1] Adipose tissue is not merely a collection of adipocytes but is an active secretory tissue releasing pro-inflammatory cytokines and suppressing anti-inflammatory mechanisms. IL-1β is particularly known for impairment of β-cell function and insulin resistance.[2] Expression of inflammatory cytokines is also increased in T2D and plays important role in development of associated microvascular and macrovascular complications. Various exogenous and endogenous factors induce immune response via intracellular receptors which are part of inflammasomes and a series of events ultimately leads to pyroptosis.

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Inflammasomes

Inflammasomes were first described by Tschopp and colleagues in 2002 and the knowledge pool has expanded till date substantially.[3] Inflammasomes are complexes of high molecular weight molecules present in cytosol of immune cells. Pattern recognition receptors (PRR) trigger assembly of inflammasomes in response to stimulus by PAMPS or DAMPS.[4] Five receptors have been identified till now and include nucleotide binding oligomerization domain (NOD), leucine rich repeat (LRR) containing NLR family members namely NLRP1, NLRP3 & NLRC4, pyrin and proteins absent in melanoma 2 (AIM2).[5] The activation mechanism and pathways of these are very well characterized. There are certain other less understood pathways stimulated via NLRP6, NLRP7, NLRP12, interferon inducible protein 16 and retinoic acid inducible gene etc. Ligand binding with these receptors results in activation and oligomerization of sensor followed by recruitment of adapter protein. NLRP3 activation/oligomerization requires a NIMA(Never in Mitosis geneA) related kinase 7 (NEK7) which gets attached to LRRs of NLRP3. Activated receptors recruit ASC adapter protein. It consists of PYD(Pyrin domain) & CARD(Caspase recruitment domain). PYD & CARD

are mandatory for recruitment of pro caspase-1 which ultimately leads to cleavage of precursor cytokines to mature cytokines initiating pyroptosis.[6]

NLRP family

Various NLRPs (Nucleotide binding oligomerization domain, LRRs and pyrins) have been identified which play role in inflammation and apoptosis. These proteins, also referred as NALP belong to NOD like receptors and are products of genes located on two chromosomes. Chromosome 11p15 contains genes for NLRP6, NLRP10 & NLRP14. NLRP1 & NLRP3 genes are present on 17p13.2 and 1q44 respectively. Rest of the NLRP genes are located on chromosome 19q13.4.[7]

Expression of NLRP genes varies across tissues. All the NLRP receptors are expressed in white blood cells. NLRP1 expression is high in nerve cells especially in pyramidal cells and oligodendrocytes. Multiple alleles of NLRP1 have been identified across various populations making it highly polymorphic gene. NLRP1, NLRP10 and NLRP3 are highly expressed in keratinocytes of epidermis. NLRP6 is mainly expressed in enterocytes where it plays role in fight against viral infections.[8]

NLRPs and inflammation:

NLRP1 is considered a key inflammasome sensor in keratinocytes which gets activated by UV radiation and is believed to play role in sunburns.[9] Mutations resulting in gain of function of NLRP1 are risk factor for developing skin cancers. It is also associated with auto-inflammation resulting in arthritis[10] and dyskeratosis[11]. Recently role of NLRP1 in pathogenesis of diabetes mellitus has been studied. It was established that NLRP1 polymorphism is associated with gasdermins (GSDMs) mediated pyroptosis in cases of T1D.[12]

NLRP3 inflammasome is under extensive study for its role in development of chronic inflammation in T2D. The caspase-1 activation results in maturation of pro-inflammatory cytokines which play important role in pathogenesis of T2D as well as development of complications.[13]

NLRP3 and its role in T2D

NLRP3 inflammasomes are activated by obesity induced danger signals in T2D patients. Studies on mice revealed palmitate-induced activation of NLRP3 leading to impaired insulin signaling and decreased glucose tolerance. Levels of chemokine 10, CCL2 and interferon-γ were reduced in NLRP3 knockout mice.[14]

Evidence suggested that level of NLRP3, IL-1β, IL-18 and ASC mRNA is elevated in patients of T2D. Studies also showed that hyperglycemia induces the expression of toll like receptors (TLR) especially TLR2 and TLR4. Raised levels of other molecules capable of activating TLR including NLRP3 are present in cases of T2D. These are high mobility group box-1 (HMGB1- high mobility group box-1) and endotoxins.[15] Hyperglycemia and oxidized LDL-cholesterol initiate priming of islet amyloid polypeptide (IAPP) in T2D, activation of NLRP3 inflammasome mediated generation of mature IL-1β.[16]

Several studies have established that expression of NLRP3 is increased inpatients of T2D. Ruscitti et al reported increased expression of NLRP3 in monocytes of T2D patients and raised levels of resultant inflammatory cytokines.[17] Similar results were later on reported by other researchers. The risk factors of T2D may also upregulate NLRP expression, thus participating in pathogenesis and development of complications. Free radicals especially reactive oxygen species (ROS) are strong activators of this inflammasome.[18] Another molecule, Thioredoxin interacting protein damages β-cells via upregulation of NLRP3.[19] Therefore, NLRP3 inflammasome seems to be a key player in pro-inflammatory environment seen in T2D patients.

NLRP3 depletion is linked with declined inflammation inT2D

Several studies were conducted in which NLRP3 or related molecules knockout animals were used to learn about their association with inflammation in T2D. Yun Hee et al established in their study that ASC & NLRP3 knockout mice have higher insulin levels compared to control group with similar conditions.[20] Other studies have revealed that IL-1β expression in adipocytes is also decreased by eliminating NLRP3.[21] Penjovic et al demonstrated that transfection of macrophages in Galectin-3(-/-) mice, administered with siRNA targeting NLRP3 resulted in altered IL-1β production.[22] All these studies reflect the strong relation between NLRP3 inflammasome activation and inflammation in T2D patients.

NLRP3 Polymorphism and T2D

Several studies investigated the association of NLRP3 polymorphism and T2D progression. It was reported that polymorphism and mutations resulting in gain in function of NLRP3 contribute to pathogenesis of T2D

and increased risk of complications. NLRP3 rs35829419 was found to be linked with vascular complications especially myocardial infarction.[23] In another study, it was reported that rs4925659 GG, rs10925027 CC, rs10754558 GG and GC+GG genotypes are at high risk of chronic inflammation and T2D.[24] A study on Indian population revealed significant association between NLRP3 GG genotype at rs10754558.[25]

Pharmacological interventions targeting NLRP3

Since the role of NLRP3 inflammasome has been established, several dietary as well as pharmacological interventions to inhibit NLRP3 have been studied. The researchers studied effects of these inhibitors on glycemic control and development of complications. The role of dietary unsaturated fatty acids in slowing down the progression of complications is established. A study by L'homme and colleagues demonstrated the key role of unsaturated fatty acids in NLRP3 inhibition among humans.[26] Similarly polyunsaturated fatty acids and omega-3 fatty acids exert their beneficial effects via NLRP3 suppression. PSPC (purple sweet potato color) derived flavonoids also inhibit NLRP3 functioning. This is associated with reduced risk of atherosclerosis in patients of T2D. Lamkanfi et al reported in their study that glyburide exerts its action by inhibition of IAPP mediated activation of NLRP3.[27] Later on Luo and colleagues established that rosuvastatin down-regulates NLRP3 expression, thus reducing inflammation in T2D patients with cardiomyopathy.[28] Various other inhibitors of NLRP3 have been investigated to treat diseases related with this inflammasome activation. These include p58, Erratum, acrylamide derivatives and tripartite motif protein 30 and promising results were reported in multiple studies.

CONCLUSION

The key role of inflammasomes in initiation and progression of inflammatory disorders is established. As T2D pathogenesis and development of complications especially vascular complications is also the result of chronic inflammation, the role of inflammasomes in DM is extensively studied. NLRP3 inflammasome activation and gain of function was found to be associated with T2D, so it was hypothesized that inhibitors of NLRP3 may be the next therapeutic option for T2D. Various inhibitors were tried in vivo and in animal studies and were found to be useful. Micro RNA based inhibition of NLRP3 was also studied. Further research in this domain may be helpful in devising new weapons for fight against T2D. The molecular knowledge of NLRP3 gene and inflammasome can be translated to design new diagnostic tools and therapeutic measures for prevention and treatment of T2D.

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