General and Comparative Endocrinology 210 (2015) 124–129

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Review

Endoplasmic reticulum stress is involved in the connection between inflammation and autophagy in type 2 diabetes

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ARTICLE INFO

Article history:
Received 31 May 2014
Revised 30 August 2014
Accepted 16 September 2014
Available online 27 September 2014

Keywords:
Type 2 diabetes
Autophagy
Inflammation
Endoplasmic reticulum stress
Interleukin 1β

ABSTRACT

Type 2 diabetes is a chronic inflammatory disease. A number of studies have clearly demonstrated that cytokines such as interleukin 1β (IL1β) contribute to pancreatic inflammation, leading to impaired glucose homeostasis and diabetic disease. There are findings which suggest that islet β-cells can secrete cytokines and cause inflammatory responses. In this process, thioredoxin-interacting protein (TXNIP) is induced by endoplasmic reticulum (ER) stress, which further demonstrates a potential role for ER stress in innate immunity via activation of the NOD-like receptor (NLRP) 3/caspase1 inflammasome and in diabetes pathogenesis via the release of cytokines. Recent developments have also revealed a crucial role for the autophagy pathway during ER stress and inflammation. Autophagy is an intracellular catabolic system that not only plays a crucial role in maintaining the normal islet architecture and intracellular insulin content but also represents a form of programmed cell death. In this review, we focus on the roles of autophagy, inflammation, and ER stress in type 2 diabetes but, above all, on the connections among these factors.

1. Introduction

Inflammation has been implicated in the pathophysiology of type 2 diabetes. However, unlike type 1 diabetes, the islet inflammatory response of type 2 diabetes is "low grade" and its role in the pathophysiology of type 2 diabetes is somewhat controversial (Imai et al., 2013). It has been shown that elevated levels of IL1β, IL6, MCP1, and C-reactive protein are predictive of type 2 diabetes (Donath and Shoelson, 2011). Further evidence supports the idea that overnutrition and insulin resistance result in the production of proinflammatory cytokines, such as IL1β, and the activation of signaling pathways that cause pancreatic β-cell death and dysfunction (Wang et al., 2013). Moreover, other research has shown that ER stress activates IL1β production by the NLRP3 inflammasome through the protein kinase RNA-like ER kinase (PERK) and inositol requiring enzyme (IRE)1 pathways and mediates β-cell death (Oslowski et al., 2012).

Additionally, an emerging body of evidence identifies autophagy as a critical modulator of the two major pathological arms of type 2 diabetes–impaired insulin secretion and insulin sensitivity. Autophagy is a cellular process that not only degrades proteins but also breaks down lipids, DNA and RNA. In this way, autophagy provides new pools of raw material for anabolic processes and drives a continuous flow of materials in a degradation-regeneration cycle within the cell (Rabinowitz and White, 2010). Autophagy may also contribute to programmed cell death, called autophagic death, which is different from apoptosis, depending on the cellular and environmental context (Tsujimoto and Shimizu, 2005). Thus, it is difficult to say whether autophagy promotes cell death or protects cells from diverse types of injuries, depending on the cellular and environmental context. Recent studies have reported that autophagy-related gene (*Atg7*) induced excessive autophagic activation in pancreatic INS-1(823/13) cells exposed to saturated fatty acids. Cathepsin B, which is induced by Atg7, contributed to the Atg7-induced NLRP3-dependent proinflammatory response and resulted in aggravation of lipotoxicity independent of apoptosis in the INS-1(823/13) cell line (Li et al., 2013). In addition, Atg16L1 is an essen-

Abbreviations: IL1β, interleukin 1β; TXNIP, thioredoxin-interacting protein; ER, endoplasmic reticulum; NLRP, NOD-like receptor; PERK, protein kinase RNA-like ER kinase; IRE, inositol requiring enzyme; *Atg7*, autophagy-related gene; LC3, light chain 3; eIF2a, eukaryotic translation initiation factor 2a; TNFα, tumor necrosis factor alpha; IFNγ, interferonγ; IKKB, inhibitors of NF-kB, kinase B; NF-kB, nuclear factor-KB; JNK, c-Jun N-terminal kinase; ROS, reactive oxygen species; *ATF*, activating transcription factor; ASC, apoptosis-associated speck-like protein containing a CARD; PA, palmitate; 3-MA, 3-methyadenine; Pdx1, pancreas duodenal homeobox 1; ULK1, UNC51-like kinase 1; PE, phosphatidylethanolamine; $Atg7^{\Delta\beta\text{-cell}}$, pancreaticβ-cell specific *Atg7*-knockout.

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http://dx.doi.org/10.1016/j.ygcen.2014.09.006
0016-6480/

tial component of the autophagic machinery responsible for regulation of endotoxin-induced IL1β production (Saitoh et al., 2008).

ER stress also plays an active role in the process of autophagy-associated management in type 2 diabetes (Bartolome et al., 2012). Although the molecular mechanism remains unclear, microtubule-associated protein 1 light chain 3 (LC3) can be converted to lipidated LC3 (LC3-II), and this process is reportedly mediated by PERK/eukaryotic translation initiation factor 2a (eIF2a) phosphorylation (Gonzalez et al., 2011). Based on the above analysis, we deduce that autophagy, inflammation and ER stress are crucial in type 2 diabetes and closely connected with each other. Thus, understanding the mechanisms of their involvement in the regulation of diabetic disease may help to identify novel therapeutic targets with important clinical applications.

2. Type 2 diabetes: a chronic inflammatory disease

Type 2 diabetes is traditionally characterized by insulin resistance/reduced systemic insulin sensitivity and islet β-cell dysfunction. The following three mechanisms in particular are associated with insulin resistance following β-cell depletion and, indirectly, immune attack: lipotoxicity, glucotoxicity, and inflammation (Odegaard and Chawla, 2012). Recently, a number of studies have distinctly demonstrated that chronic tissue inflammation is a key contributing factor to type 2 diabetes. Elevated levels of glucose and lipids, particularly saturated fatty acids, are characteristics of insulin resistance and synergize within the β-cell to drive parallel increases in FAS expression (Unger, 1995). This contributes to the pathogenesis of type 2 diabetes via ER stress and the generation of reactive oxygen species, both of which culminate in inflammatory cytokine secretion (Harding and Ron, 2002; Hotamisligil, 2010). In particular, IL1β secretion has been known to be a mediator of β-cell dysfunction and death for more than 25 years, and its effects are potentiated by tumor necrosis factor alpha (TNFα); interferonγ (IFNγ) (Mandrup-Poulsen et al., 1985; Pukel et al., 1988; Hotamisligil, 2010). Additionally, a recent study indicated that IL6 and IL10 are important physiological contributors to the central insulin and leptin actions mediated by exercise, thus linking to hypothalamic ER stress and inflammation. The impairment of hypothalamic insulin and leptin signaling pathways is sufficient to promote hyperphagia, obesity, and T2D. This study also showed that the activation of inhibitors of NF-kB, kinase B (IKKB)/nuclear factor-KB (NF-kB) through elevated endoplasmic reticulum stress in the hypothalamus is associated with central insulin resistance. In this process, examination of ER stress markers demonstrated increased levels of PERK phosphorylation and of c-Jun N-terminal kinase (JNK) and IKKB activity (Ropelle et al., 2010).

The most noteworthy feature of type 2 diabetes, unlike type 1 diabetes, is that the islet inflammatory response is "low-grade" and its role in the pathophysiology of type 2 diabetes is somewhat controversial. The latest research shows NKp46 is involved in the killing of murine β-cells in type 1 diabetes (Imai et al., 2013). However, a totally different argument could be made in type 2 diabetes. Insulin depletion in diabetic β-cells may protect them from NK cell attack and thus should be viewed as a protective response, thereby preventing NKp46-mediated β-cell destruction during low-grade inflammation (Gur et al., 2013).

3. ER stress is involved in inflammation in the pathogenesis of type 2 diabetes

A chronic excess of metabolic factors (such as lipids, glucose and cytokines), intracellular calcium and free radicals [such as reactive oxygen species (ROS) and nitric oxide] can trigger not only inflammation but also ER stress, which further disrupts metabolic functions and thereby causes more inflammation (Zhang and Kaufman, 2008). However, this cycle of inflammation is transient and NF-kB-dependent (Böni-Schnetzler et al., 2008). Under such conditions, failure of the ER's adaptive capacity results in activation of the UPR (Hotamisligil, 2010). Moreover, during ER stress, excess accumulation of unfolded or misfolded proteins causes cells to consume extra reduced glutathione to correctly fold these aberrantly assembled proteins, thus adding to cellular stress. Consequently, ER stress can lead to oxidative stress, which might trigger an inflammatory state, as discussed earlier (Alfadda and Sallam, 2012).

Recently, Oslowski et al. showed that TXNIP (an early response gene highly induced by diabetes and hyperglycemia, encoding an endogenous inhibitor of the antioxidant thioredoxin) is a critical signaling node that links ER stress and inflammation. TXNIP has a demonstrated potential role in innate immunity during diabetes via activation of the NLRP3 inflammasome and release of IL1β, which mediates oxidative stress and ER-stress-mediated β-cell death (Zhou et al., 2010; De Nardo and Latz, 2011; Oslowski et al., 2012). These data have demonstrated that the transcriptional expression of TXNIP is regulated by *ChREBP* and activating transcription factor *(ATF) 5*. It has been shown that *ChREBP* expression was induced by ER stress and significantly decreased in *PERK* knockdown INS-1 832/13 cells and *PERK* knockout mouse embryonic fibroblasts. Additionally, it has been shown that PERK-mediated eIF2α phosphorylation directs the protein translation and mRNA transcription of *ATF5* and that *ATF5* is integral to the eIF2α kinase response. In *Ire1α/PERK* knockdown INS-1 832/13 cells and MEFs, TXNIP expression was modestly attenuated compared to control cells under ER stress conditions. In conclusion, the expression of TXNIP induced by ER stress is under the control of the IRE1α and PERK-eIF2α pathways of the UPR. This experiment proved that IL1β and IL6 upregulation was attenuated in TXNIP knockdown cells compared to control cells (Oslowski et al., 2012). Moreover, TXNIP binds to and inhibits thioredoxin and thereby can modulate the cellular redox state and promote oxidative stress (Patwari et al., 2006). These findings support the conclusion that a variety of stress signaling pathways converge at TXNIP and lead to inflammasome activation and IL1β production. IL1β is usually involved in the activation of a protein complex termed the inflammasome. The NLRP subfamily (NLRP1, NLRP3 and NLRC4), and the PYHIN family protein absence in melanoma 2 have been shown to form inflammasomes, which are high molecular weight signaling platforms. Among these is NLRP3, which contains the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase1. Activation induces oligomerization of the NLRP3 inflammasome and recruits ASC through homotypic PYD–PYD interaction. Then, ASC recruits pro-caspase1 leading to autocatalytic activation of caspase1, and the active caspase1 hetero-tetramers are able to convert inactive pro-IL1β and pro-IL18 into their bioactive and secreted forms (De Nardo and Latz, 2011). Patterns or danger-associated molecular patterns activate the inflammasome. Once IL1β leaves the cell, it binds to the IL1 receptor and causes inflammation. Both potassium (K+) efflux and an increase in ROS are necessary for the activation of the NLRP3 inflammasome in response to all stimuli tested thus far. Recent studies suggest that ER stress, like other NLRP3 activators, activates the NLRP3 inflammasome in a K+ efflux- and ROS-dependent manner that may also affect the mitochondria (Menu et al., 2012). Hence, we infer that ER stress initiates a signal that is transmitted to mitochondria and then relayed to the NLRP3 inflammasome.

Inflammatory cytokines converge on IKKB (an inhibitor of the kappa light polypeptide gene enhancer in β-cells), and mitogen-activated protein kinase 8/JNK1 to directly inhibit insulin action via serine phosphorylation of insulin receptor substrate 1 and 2 (Odegaard and Chawla, 2012). β-cell damage and concomitant