Thioredoxin-Interacting Protein Is Killing My β-cells!

John A. Corbett

The oxidation of glucose is required for the secretion of insulin by pancreatic β-cells. The product of this oxidation, ATP, stimulates the closure of potassium channels and calcium-dependent exocytosis of insulin. This system is beautifully regulated such that the precise amount of insulin is delivered to the bloodstream to regulate whole-body glucose metabolism. On the other hand, when insulin fails to function properly or when the demand for insulin is greater than the levels released by β-cells, blood glucose concentrations rise and diabetes ensues. In a subset of individuals with type 2 diabetes, there is a significant loss of β-cell mass. The mechanisms by which β-cells are lost under conditions in which glucose levels are elevated have yet to be fully elucidated. Proposed mechanisms to explain β-cell toxicity to elevated glucose concentrations include (but are not limited to) the generation of free radicals in β-cells during oxidative metabolism of glucose (1), the production of proinflammatory cytokines such as interleukin-1, which are known to be toxic to β-cells (2), and the induction of endoplasmic reticulum (ER) stress in β-cells (3). It has been proposed that each of these damaging pathways results in loss of β-cells due to apoptosis. In the current issue of *Diabetes*, Chen et al. (4) provide evidence that the loss of β-cell mass in response to elevated concentrations of glucose is the results of enhanced expression of a single protein, thioredoxin-interacting protein (TXNIP).

TXNIP participates in control of the redox state of a cell by interacting and inhibiting thioredoxin (5). Thioredoxin contains conserved active-site cysteine residues that are reversibly reduced by NADPH-dependent thioredoxin reductase. TXNIP has been identified as one of the most highly inducible genes in human islets treated with elevated glucose levels (6). Glucose-stimulated TXNIP expression, which does not require glucose metabolism or new protein synthesis, is regulated by a carbohydrate response element that consists of two E-box elements in the human TXNIP promoter (7). Those at the Shalev laboratory (7) also have shown that stable overexpression of human TXNIP in a β-cell line results in an increase in the Bax-to-*Bcl*II ratio, enhanced expression of caspase 3, and cleavage of caspase 9 to its active form (all proapoptotic events). Now, Chen et al.,(4) extend these initial observations to show that TXNIP expression is increased by approximately eightfold in islets isolated from diabetic (8-week-old) BTBR.ob mice ompared compared with that in islets isolated from BTBR.lean controls and that TXNIP expression correlates with an approximately eightfold increase in caspase 3 cleavage. These findings correlate enhanced expression of TXNIP with increased caspase 3 cleavage and diabetes in a mouse model.

To provide evidence that enhanced TXNIP expression mediates the loss of β-cell viability, caspase 3 cleavage and DNA damage, as assessed by transferase-mediated dUTP nick-end labeling staining, were examined using islets isolated from TXNIP-deficient mice (HcB-19 [8]) or mice with the targeted deletion of TXNIP. As predicted by the authors, glucose does not enhance caspase 3 cleavage or DNA damage in islets isolated from HCB-19 (TXNIP-deficient) mice or mice with a targeted deletion of TXNIP. The mechanism by which enhanced expression of TXNIP mediates β-cell death appears to be induction of the intrinsic apoptotic cascade. Expression of TXNIP under control of the rat insulin promoter in a β-cell line is associated with the release of cytochrome C from the mitochondria. Prolonged activation of ER stress due to the high levels of forced expression of proteins has been shown to cause death by apoptosis (9), and elevated levels of glucose have been shown to induce ER stress (10) and β-cell apoptosis (2). In contrast to these potential mechanisms of apoptosis induction, the authors clearly show that overexpression of TXNIP in β-cells does not result in an increase in the expression of either Bip (immunoglobulin heavy-chain binding protein) or Chop (C/EBP homologous protein), two markers of ER stress. The β-cell maintains a highly adaptive unfolded protein response due to its function as a professional secretory cell (9), and prolonged ER stress has been implicated in the demise of β-cells in response to lipids (11), glucose, and cytokines (3). Consistent with the authors' conclusions, recent reports indicate that β-cell death in response to cytokines does not depend on the induction of ER stress (12).

In many cases, the discovery of a novel pathway or mechanism of action encourages more questions than those answered by a given study. The novel role of TXNIP as a potential regulator of β-cell mass is no exception. Importantly, the authors have associated enhanced expression of TXNIP with caspase 3 cleavage in islets isolated from diabetic mice; however, is the enhanced expression of TXNIP a cause of diabetes development (or loss of β-cell mass) or a consequence of diabetes? Studies using the recently generated Cre-loxP mouse with targeted deletion of TXNIP in β-cell will likely prove to be an extremely useful tool to address this question, either by crossing this mutant onto a mouse line that spontaneously develops type 2 diabetes or by examining the effects of a secondary stress such as obesity induced by a high-fat diet on diabetes development and regulation of β-cell mass. Glucose, the primary stimulus for insulin secretion, seems

From the Department of Medicine, the Comprehensive Diabetes Center, University of Alabama in Birmingham, Birmingham, Alabama.

Address correspondence and reprint requests to John Corbett, Shelby Building, 12th floor, 1530 3rd Ave. So., UAB, Birmingham, AL 35294. E-mail: corbettj@uab.edu.

Received and accepted for publication 14 January 2008.

ER, endoplasmic reticulum.

DOI: 10.2337/db08-0055

© 2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Please see accompanying original article, p. 938

to be the inducer of TXNIP expression. If TXNIP proves to be the mediator of β-cell death in response to elevated levels of glucose, there will clearly be an important regulatory mechanism to be identified that allows for normal stimulus secretion coupling in response to glucose and apoptosis of β-cells under conditions of glucotoxicity. Third, the mechanisms responsible for controlling mitochondrial cytochrome c release in β-cells expressing high levels TXNIP need to be elucidated. It is intriguing to speculate that the proapoptotic actions of TXNIP may be associated with its ability to interact and inhibit thioredoxin (5). Under conditions of cellular stress (H_2O_2 and ultraviolet light exposure), there is an increase in the expression of thioredoxin as a defense mechanism to alleviate the toxic stress (13). In addition, transgenic expression of thioredoxin in β-cells attenuates diabetes development in the nonobese diabetic mouse and diabetes induced by streptozotocin (14). Are the underlying mechanisms responsible for the death of cells expressing TXNIP associated with the sequestering of thioredoxin and the inhibition of thioredoxin-mediated protection? Perhaps a more intriguing yet speculative issue concerns the role that TXNIP plays in the regulation of β-cell mass. The authors state that transgenic mice deficient in TXNIP (HcG-19) have increased pancreatic insulin content (suggesting enhanced β-cell mass) with rates of β-cell proliferation comparable with those of control mice. Does TXNIP participate in the remodeling phase of islet development that occurs shortly after birth? Further, Mathis and coworkers have proposed that β-cell apoptosis during islet remodeling results in the release of antigens that direct autoimmunity against β-cells (15). If the absence of TXNIP prevents the loss of β-cell mass during islet remodeling, studies designed to examine the effects of TXNIP deficiency on the development of autoimmune diabetes in the NOD mouse could be very intriguing.

In summary, the work of Chen et al. (4) provides interesting experimental evidence suggesting that glucose toxicity is not due to prolonged ER stress but, rather, enhanced expression of a single protein, TXNIP. This is a novel observation that will likely simulate new studies focused on the mechanisms by which enhanced TXNIP expression drives apoptosis, how the absence of this thioredoxin-interacting protein protects against apoptosis, and other potential roles of this novel redox active protein in the survival and death of pancreatic β-cells.

REFERENCES

1. Robertson RP: Oxidative stress and impaired insulin secretion in type 2 diabetes. *Curr Opin Pharmacol* 6:615–619, 2006
2. Maedler K, Donath MY: Beta-cells in type 2 diabetes: a loss of function and mass. *Horm Res* 62:67–73, 2004
3. Eizirik DL, Cardozo AK, Cnop M: The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev* 29:42–61, 2008
4. Chen J, Saxena G, Mungrue IN, Lusis AJ, Shalev A: Thioredoxin-interacting protein: a critical link between glucose toxicity and β-cell apoptosis. *Diabetes* 57:938–944, 2008
5. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, Nakamura H, Takagi Y, Sono H, Gon Y, Yodoi J: Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. *J Biol Chem* 274:21645–21650, 1999
6. Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, Brady JN, Harlan DM: Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. *Endocrinology* 143:3695–3698, 2002
7. Minn AH, Hafele C, Shalev A: Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology* 146:2397–2405, 2005
8. Hui TY, Sheth SS, Diffley JM, Potter DW, Lusis AJ, Attie AD, Davis RA: Mice lacking thioredoxin-interacting protein provide evidence linking cellular redox state to appropriate response to nutritional signals. *J Biol Chem* 279:24387–24393, 2004
9. Wu J, Kaufman RJ: From acute ER stress to physiological roles of the unfolded protein response. *Cell Death Differ* 13:374–384, 2006
10. Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, Filipponi F, Weir GC, Eizirik DL, Cnop M: The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia* 50:2486–2494, 2007
11. Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, Biden TJ: Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* 50:752–763, 2007
12. Chambers KT, Unverferth JA, Weber SM, Wek RC, Urano F, Corbett JA: The role of nitric oxide and the unfolded protein response in cytokine-induced beta-cell death. *Diabetes* 57:124–132, 2008
13. Holmgren A: Antioxidant function of thioredoxin and glutaredoxin systems. *Antioxid Redox Signal* 2:811–820, 2000
14. Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, Yodoi J, Miyazaki J: Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J Exp Med* 188:1445–1451, 1998
15. Turley S, Poirot L, Hattori M, Benoist C, Mathis D: Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *J Exp Med* 198:1527–1537, 2003