Licio A. Velloso

Turning Off a Viral/Lipid Sensor Improves Type 2 Diabetes

Diabetes 2014;63:393–395 | DOI: 10.2337/db13-1704

Type 2 diabetes (T2D) results from the combination of insulin resistance and a relative deficiency of insulin secretion (1,2). Over the last 20 years, clinical and experimental studies have advanced our understanding of the cause of insulin resistance, revealing the existence of subclinical inflammation in subjects with increased adiposity (3,4). Inflammatory cytokines produced in the context of this metabolic inflammation activate serine/threonine kinases that target proteins of the insulin signaling pathway, rendering them unresponsive to the insulin signal (5,6). Unfortunately, despite great advances obtained in the characterization of the pathophysiology of T2D, no anti-inflammatory approaches have been developed as therapeutic options for this disease.

Excessive nutrient consumption, particularly fatty acids, is regarded as one of the main triggers of metabolic inflammation (7). Among the mechanisms identified as a link between dietary fats, inflammation, and insulin resistance, endoplasmic reticulum (ER) stress has received special attention; it occupies a pivotal position, as it simultaneously acts as a target and a trigger of a number of inflammatory and metabolic events that are closely related to insulin resistance (8,9). On the one hand, ER stress can be induced by either excessive nutrient load (10) or extracellular inflammatory signals (11); alternatively, the unfolded protein response generated to reestablish ER homeostasis can activate inflammatory signaling proteins, such as Jun NH2-terminal kinase (JNK) and inhibitory κB kinase (IKK), thereby inducing transcription of inflammatory genes, such as *Tnfa* and *Il6* (10).

A recent study provided an important advance in this field when it identified the double-stranded RNA–dependent kinase (PKR) as a protein that integrates inflammation, nutrient overload, and ER stress (12). PKR was originally described as an intracellular sensor of viral infection (13), whose activation leads to the simultaneous induction of an inflammatory response against the infecting agent and a reduction of protein synthesis aimed at inhibiting viral proliferation (14). As the molecular actions of PKR intersect with pathways involved in insulin resistance, it was hypothesized that PKR could act as a sensor of not only viral infection but also of nutrient overload (12). In fact, PKR is activated in metabolically relevant tissues in obesity as well as when lipids are infused in lean mice. Once active, PKR can directly and indirectly (through JNK) target insulin receptor substrate-1, leading to its serine phosphorylation, thereby rendering it unresponsive to insulin (12). In addition, a recent study has shown that PKR is active in the liver, skeletal muscle, and white adipose tissue of obese humans, and that body mass reduction resulting from bariatric surgery is accompanied by a reduction of PKR activity (15). In at least two studies (12,16), genetic approaches aimed at reducing PKR activity resulted in considerable improvement in a number of metabolic parameters related to obesity and T2D. Thus, because of its nodal position in which it integrates infectious, inflammatory, and metabolic signals, PKR emerges as a potential target for pharmacological treatment of diabetes.

In this issue, Nakamura et al. (17) tested two small molecules that act as chemical inhibitors of PKR—imoxin and 2-aminopurine—for their efficacy to reduce the diabetes phenotype of *ob/ob* mice. They showed that pharmacological inhibition of PKR resulted in improved insulin signal transduction and improved glucose tolerance, which were accompanied by a reduction in obesity-associated inflammatory activity in adipose tissue.

Imoxin is an imidazolo-oxindole inhibitor of PKR that was identified during the screening of a library of compounds evaluated for their capacity to bind and inhibit the ATP-binding site of PKR (18). In a recent study, imoxin efficiently inhibited the PKR-dependent

Laboratory of Cell Signaling, Obesity and Comorbidities Research Center, University of Campinas, Campinas, Brazil

Corresponding author: Licio A. Velloso, lavelloso.unicamp@gmail.com.

See accompanying original article, p. 526.

activation of inflammasome, thereby illustrating its potential use as an anti-inflammatory drug (19). Another molecule, 2-aminopurine, is an analog of guanine and adenine and a well-known inhibitor of PKR. Although not structurally related to imoxin, it also acts by inhibiting the ATP-dependent activation of PKR (20).

A number of distinct genetic and pharmacological approaches aimed at reducing inflammation have been tested in the treatment of experimental T2D, with widespread success (8,9). Targets such as tumor necrosis factor-α (TNFα), JNK, IKK and the SOCS-3 gene were all efficiently inhibited in animal models, resulting in an improved metabolic phenotype (2,8,9). However, more than 20 years after the first descriptions of the pathogenetic role of inflammation in T2D, we have yet to see an anti-inflammatory drug available as an option to treat diabetic humans. Currently, only three classes of drugs are recommended by the American Diabetes Association as pharmacological options for treating people affected by T2D: metformin, insulin, and inhibitors of glucagon-like peptide 1 (21).

Studies have been conducted to evaluate the effects of anti-TNFα and anti–interleukin-1β therapy in T2D. The results are controversial, with some describing significant improvements in glucose control and others reporting no metabolic benefits (22–24). However, the main problem here, particularly concerning anti-TNFα therapy, appears to be the complications that may arise over the course of prolonged use. These include tuberculosis and some types of malignancies (23,25). Perhaps more promising are the results obtained using salsalate, which is a modified form of salicylate that acts as an inhibitor of IKK. A recent trial reported improvements in blood glucose control without major side effects, although the actual reduction of the HbA_{1c} level was less than 1% (26).

With the findings of these studies in mind, what would be the advantage of inhibiting PKR over the other anti-inflammatory approaches that have already been tested? At the mechanistic level, PKR lies at the interface between metabolic and inflammatory pathways. It acts as a sensor for nutrients and integrates the incoming signal with intracellular inflammatory pathways, the insulin signaling pathway, and protein/lipid synthesis in the ER (Fig. 1). The drugs currently in use for T2D, including metformin, insulin, and inhibitors of glucagon-like peptide 1 signaling, act primarily on the metabolic side of this problem, whereas the drugs tested, with limited success, for inhibiting TNFα, interleukin-1β, and IKK act primarily on the inflammatory side. Therefore, it could be anticipated that a drug that simultaneously modulates the metabolic and inflammatory aspects of T2D would represent a therapeutic advantage. Concerning safety, inhibiting some inflammatory pathways may impact general immune surveillance, raising concerns about the risk/benefits of clinical use. Compared with the impact of inhibiting

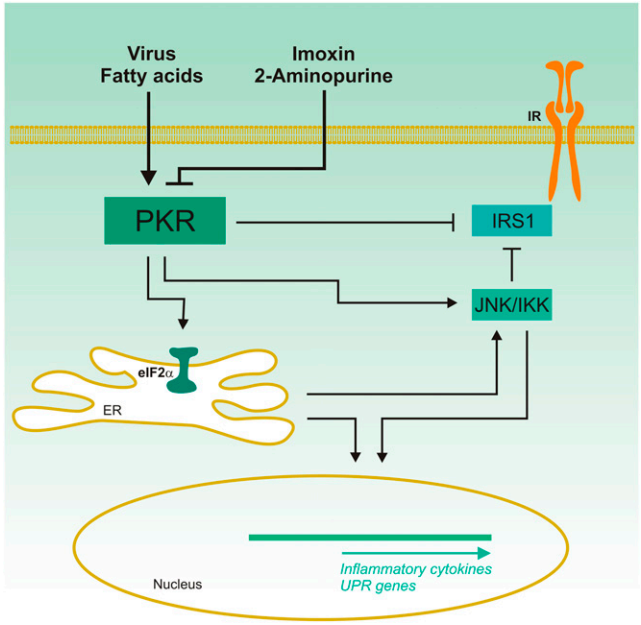

Figure 1—PKR is at the confluence of inflammatory and metabolic signals. Infectious agents (virus) and nutrients (fatty acids) can activate PKR, which, in turn, directly and indirectly activate inflammatory activity. Inflammatory activity can be triggered by the direct action of PKR on JNK or indirectly through the induction of ER stress triggered by the phosphorylation of complex eukaryotic initiation factor 2 α (eIF2α). ER stress boosts inflammation through the activation of JNK and IKK and the activation of inflammatory gene transcription. PKR, JNK, and IKK negatively target insulin receptor substrate-1, reducing the activity of insulin. The small molecules imoxin and 2-aminopurine inhibit PKR, resulting in the relief of inflammation and insulin resistance. IR, insulin receptor; IRS-1, insulin receptor substrate-1; UPR, unfolded protein response.

inflammatory cytokines, targeting PKR has been reported to be quite safe in experimental animals, as no important immunological defects have been observed. In humans, no major clinical outcomes are related to the genetic variants of the *Pkr* gene. However, a few reports have linked somatic *Pkr* mutations to leukemia (27,28). Therefore, preclinical studies should be performed to evaluate the impact of the long-term chemical inhibition of PKR. An additional potential advantage offered by Nakamura et al. (17) is that PKR inhibition was achieved using small molecules. In a number of diseases, identification of potential therapeutic targets can fall short because of the inability to produce drugs with practical clinical use. Here, we have a case in which the identification of PKR as a potential target for T2D only two years ago was rapidly followed by experimental tests with small molecules that, at least in theory, may offer a tremendous advantage for clinical use.

As the prevalence of obesity and T2D continues to increase, identification of novel targets for therapy renews the hope for safer and more efficient therapeutic methods. The inhibition of PKR may represent the first

attempt to simultaneously tackle distinct pathways that are intimately related to the development of T2D.

Funding. The Laboratory of Cell Signaling and the Obesity and Comorbidities Research Center are supported by grants from Sao Paulo Research Foundation and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802–1812
2. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–1801
3. Schmidt MI, Duncan BB, Sharrett AR, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999;353:1649–1652
4. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
5. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333–336
6. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ. Science 2001;293:1673–1677
7. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol 2008;8:923–934
8. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415–445
9. Cnop M, Foufelle F, Velloso LA. Endoplasmic reticulum stress, obesity and diabetes. Trends Mol Med 2012;18:59–68
10. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457–461
11. Coope A, Milanski M, Arruda AP, et al. Chaperone insufficiency links TLR4 protein signaling to endoplasmic reticulum stress. J Biol Chem 2012;287: 15580–15589
12. Nakamura T, Furuhashi M, Li P, et al. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell 2010;140:338–348
13. Samuel CE. Antiviral actions of interferon. Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. Virology 1991; 183:1–11
14. Takada Y, Ichikawa H, Pataer A, Swisher S, Aggarwal BB. Genetic deletion of PKR abrogates TNF-induced activation of IκBα kinase, JNK, Akt and cell proliferation but potentiates p44/p42 MAPK and p38 MAPK activation. Oncogene 2007;26:1201–1212
15. Carvalho BM, Oliveira AG, Ueno M, et al. Modulation of double-stranded RNA-activated protein kinase in insulin sensitive tissues of obese humans. Obesity (Silver Spring) 21 March 2013 [Epub ahead of print]
16. Carvalho-Filho MA, Carvalho BM, Oliveira AG, et al. Double-stranded RNA-activated protein kinase is a key modulator of insulin sensitivity in physiological conditions and in obesity in mice. Endocrinology 2012;153:5261–5274
17. Nakamura T, Arduini A, Baccaro B, Furuhashi M, Hotamisligil GS. Small-molecule inhibitors of PKR improve glucose homeostasis in obese diabetic mice. Diabetes 2014;63:526–534
18. Jammi NV, Whitby LR, Beal PA. Small molecule inhibitors of the RNA-dependent protein kinase. Biochem Biophys Res Commun 2003;308:50–57
19. Lu B, Nakamura T, Inouye K, et al. Novel role of PKR in inflammasome activation and HMGB1 release. Nature 2012;488:670–674
20. Hu Y, Conway TW. 2-Aminopurine inhibits the double-stranded RNA-dependent protein kinase both in vitro and in vivo. J Interferon Res 1993; 13:323–328
21. American Diabetes Association. Standards of medical care in diabetes—2013. Diabetes Care 2013;36(Suppl. 1):S11–S66
22. Gupta-Ganguli M, Cox K, Means B, Gerling I, Solomon SS. Does therapy with anti-TNF-αimprove glucose tolerance and control in patients with type 2 diabetes? Diabetes Care 2011;34:e121
23. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFα therapy. Scand J Rheumatol 2007;36:91–96
24. Larsen CM, Faulenbach M, Vaag A, et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007;356:1517–1526
25. Targownik LE, Bernstein CN. Infectious and malignant complications of TNF inhibitor therapy in IBD. Am J Gastroenterol 17 September 2013
26. Goldfine AB, Fonseca V, Jablonski KA, et al.; Targeting Inflammation Using Salsalate in Type 2 Diabetes Study Team. Salicylate (salsalate) in patients with type 2 diabetes: a randomized trial. Ann Intern Med 2013; 159:1–12
27. Hii SI, Hardy L, Crough T, et al. Loss of PKR activity in chronic lymphocytic leukemia. Int J Cancer 2004;109:329–335
28. Murad JM, Tone LG, de Souza LR, De Lucca FL. A point mutation in the RNA-binding domain I results in decrease of PKR activation in acute lymphoblastic leukemia. Blood Cells Mol Dis 2005;34:1–5