Cytokine-Induced Dicing and Splicing in the β-Cell and the Immune Response in Type 1 Diabetes

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Cytokines play a prominent role in mediating the inflammatory response related to injury, infection, and physiological processes from reproduction (1) to suntanning (2). Their impact on the target tissue can be destructive or protective depending on local concentrations, the spectrum of cytokine responses, and accompanying contact-mediated cellular inflammatory processes (3,4). In this issue of *Diabetes*, Ortis et al. (5) examine the transcriptional response of isolated primary β-cells after 6 and 24 h to mixtures of interleukin (IL)-1β and interferon (IFN)-γ or tumor necrosis factor (TNF)-α and IFN-γ under conditions culminating in extensive apoptosis by 72 h. None of these cytokines individually cause apoptosis, and the objective was to define a transcriptional inflammatory signature that links their combination to oxidative stress, endoplasmic reticulum (ER) stress, and cell death.

Upwards of 20% of the β-cell transcriptome is altered by these cytokines, resulting in deterioration in the function of the cell and a reversal of the β-cell phenotype toward a dedifferentiated state. The authors observe downregulation of Krebs cycle enzyme transcripts that could impact oxidative phosphorylation and stimulus secretion coupling, downregulation of transcription factors involved in β-cell lineage determination and insulin gene transcription, and downregulation of incretin and hormone receptor transcripts that modulate β-cell mass in response to diet and pregnancy. By contrast, the production of cytokines and chemokines by β-cells through a synergistic effect of TNFα and interferon signaling on IRF-7 seems to tell a different story. It fits with the authors hypothesis of a dialogue among the cellular elements affected by viral infection or immune attack that may act to amplify or squelch the local inflammatory response (6). Are we witnessing the death knell of a cell destined to undergo apoptosis or an act of self-preservation through energy conservation and a call for help?

In a parallel experiment the authors evaluated alternative splicing of pancreatic β-cell transcripts using Affymetrix Rat Exon 1.0 ST microarrays. Some 3,000 genes, one fifth of the rat β-cell transcriptome, showed alternative splicing. More remarkably, around 300 of these exhibited changes in the relative expression of splice variants in response to cytokines. These included inducible nitric oxide synthase (iNOS) (Δ exon 8), argininosuccinate synthetase (Δ exon 1), and NFKB2 (Δ exon 22), three of the primary downstream targets of IL-1β and TNF that impact biochemical pathways leading to nitric oxide (NO) production. Previous studies have documented four common splice variants of human iNOS that show differential tissue-specific expression and are inducible by cytokines and lipopolysaccharide (7). Because homodimerization of iNOS is essential for enzyme activity, heterodimer formation between the alternatively spliced variants may regulate iNOS kinetics. The relative and absolute changes in the splice variants of the three target genes in β-cells were extensive, dynamic, and differentially regulated by the cytokine cocktail (see Fig. 7 in the accompanying article). By contrast, changes in a panel of 20 gene transcripts related to the splicing machinery were modest, arguing against global dysregulation of splicing and suggesting the existence of yet-to-be-identified regulatory elements.

The ability of cytokines to induce alternate splicing in purified β-cells has broader ramifications for the development of autoimmunity in type 1 diabetes. The islet autoantigen (IA)-2, a transmembrane protein of insulin secretory granule, is transcribed and translated as a shorter Δ exon 13 variant (8). This results in a 73aa in-frame deletion including its transmembrane domain and subsequent secretion of IA-2. In the thymus only the Δ exon 13 form is found (9), which correlates with lack of immune tolerance to T-cell and B-cell epitopes encoded by exon 13 in type 1 diabetes (10). The islet autoantigen islet-specific glucose-6-phosphatase–related protein (IGRP) (11) is another example for which different splice variants are expressed in islet and the thymus (12). Five of seven IGRP splice variants disrupt the reading frame and likely alter the topology of this nine-transmembrane ER protein. Alternate splicing of IGRP might also give rise to enhanced self-antigen presentation of MHC class I epitopes through immunoribosome-based surveillance (13). A survey of 45 autoantigens associated with other autoimmune disorders showed that all were subject to alternative splicing compared with 42% in a reference set (14) and that 80%, like IGRP (15), show noncanonical splicing compared with 1% in the nonantigen population.

Alternative splicing, in addition to regulating the β-cell proteome, may also play a critical role in the maintenance of peripheral immune tolerance. Peripheral tolerance depends upon the expression of tissue-specific antigens in secondary lymphoid tissues in a manner that triggers functional deletion of autoreactive T-cells. The autoimmune regulator (AIRE) protein is the best known transcriptional regulator of this process (16); however, a second, independent regulator Deaf1 was recently identified (17). A Deaf1 splice variant acts as a dominant inhibitor of the wild-type protein and is upregulated in the pancreatic-draining lymph nodes of pre-diabetic NOD mice and subjects with type 1 diabe-

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tes. Yet another class of splice variant associated with autoimmunity is that involved in immune recognition and regulation of T-cell viability including PD-1 (18), FAS (19), CD45 (20), and the T-cell receptor ζ chain (21).

The specific experimental model used here may be of greater relevance to the cytokine storm that accompanies acute rejection of islet transplants (22) than the slow and specific attrition of β-cells in type 1 diabetes. Nevertheless, many of the same cytokines are involved including the primary assailants produced by T-cells, macrophages, and antigen-presenting cells. The downstream network of cytokines and chemokines produced by the β-cells is potentially the same, but the islet in autoimmunity is also likely to encounter protective cytokines arising from regulatory T-cells in the lesion and other counterregulation from within the islet and beyond. Cytokine-mediated alternative splicing now clearly emerges as a potential regulatory mechanism and one that can operative on different time scales depending on mRNA and protein stability. It could certainly amplify the autoimmune response through generation of neoantigens and epitope spreading in existing β-cell immune targets. It is worth considering that similar processes might be at work also in response to inflammation triggered by infection, gluco-lipotoxicity (23), or a β-cell toxin like streptozotocin, which when used in low doses induces an immune-like destruction of β-cells (24).

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