Autoimmune T cell recognition of alternative-reading-frame-encoded peptides

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A recent study shows that a self-peptide generated in pancreatic islet beta cells through the translation of a noncanonical alternative reading frame in human insulin mRNA is recognized by both CD4+ and CD8+ T cells in type 1 diabetes.

Type 1 diabetes (T1D) results from the catastrophic loss of pancreatic islet beta cells, which are the sole source of insulin, an essential hormone for glucose homeostasis. The destruction of beta cells by $CD8^+$ T cells is now clearly implicated in the pathogenesis of T1D—but what do autoimmune T cells recognize, and why do normal immune-tolerance mechanisms fail? In this issue of *Nature Medicine*, Kracht *et al.*[1] reveal the contribution of defective ribosomal products (DRiPs) to T1D in the form of an autoimmunogenic major histocompatibility complex (MHC) class I peptide ligand that is translated from a reading-frame-shifted sequence in insulin mRNA[1]. This provides the first evidence for the participation of noncanonical translation, and more generally, DRiPs, in autoimmunity.

$CD8^+$ T cells recognize oligopeptides bound to cell-surface MHC class I molecules. DRiPs were conceived originally as a possible means by which MHC class I peptide ligands could be rapidly generated from otherwise highly metabolically stable viral proteins[2]. This idea proposed that inevitable, and perhaps deliberate[3], errors in the transcription and translation of viral genetic information generates a pool of rapidly degraded polypeptides that enable $CD8^+$ T cells to recognize and kill virus-infected cells before progeny virions are produced. DRiPs seem to be the principal source of viral peptides, and are known to provide a sizeable fraction of self-peptides.

There is mounting evidence that noncanonical translation[4]—including CUG-based initiation[5], downstream initiation in standard and altered reading frames[6], stop-codon read through[7], translation of intronic sequences[8], and translation associated with nonsense-mediated mRNA decay[9]—contributes self and foreign class I peptide ligands for $CD8^+$ T cell immunosurveillance of viruses, tumor cells and allogeneic transplants. Furthermore, ribosome profiling, which employs deep sequencing of ribosome-protected mRNA footprints, reveals extensive translation of mRNA sequences that were previously thought to be untranslated, and has thus provided new rules for translation initiated by a non-AUG codon[10]. Because noncanonical translation is likely to be enhanced by genetic mutations and inflammation-induced stress—as might occur in cancer and autoimmunity—the resulting translation products could selectively escape central and peripheral immune-tolerance mechanisms that function to prevent the generation of autoimmune T cells or to silence their activity. Indeed, inflammation is a hallmark of T1D and other autoimmune diseases.

Here Kracht *et al.*[1] reason that the massive translation of insulin by beta cells, which accounts for up to 15% of beta cell protein mass, increases the odds of nonphysiological translation of alternative reading frames under inflammatory stress. They thus searched for potentially immunogenic products encoded by an alternative reading frame. Through sequence analysis, they found that insulin mRNA encodes a polypeptide (INS-DRiP) in a reading frame that overlaps the one that encodes insulin, shifted by two nucleotides—that is, in the +2 reading frame—creating a completely novel protein, and is potentially initiated by a downstream AUG codon (**Fig. 1**). INS-DRiP is unlikely to encode a functional protein because, when lacking a stop codon, translation should continue into the poly-A tail, generating poly-Lys (decoded from AAA triplets) after a sequence too short (43 amino acids) to fold into a stable protein. By using GFP reporters in cell lines, the authors showed that the predicted chimeric protein is translated, and further, that INS-DRiP translation is enhanced by thapsigargin, a drug that induces a cellular stress response by reducing calcium levels in the endoplasmic reticulum (ER), causing misfolding of ER-targeted proteins.

The authors found that a version of the 43-mer INS-DRiP synthesized in bacteria was able to elicit immune responses *in vitro* from $CD4^+$ T cells from a patient with T1D, and that these responses were of similar magnitude to those elicited by previously identified T1D-associated antigens. To identify the exact epitope responsible for eliciting the immune response, the authors fed INS-DRiP to dendritic cells—specialized immune cells adept at activating naive T cells—and analyzed the peptides recovered from human leukocyte antigen (HLA)-class II DQ molecules (DQ, DR and DP are the three types of human class II molecules that function to present peptides to $CD4^+$ T cells). They identified a 9-mer peptide corresponding to the INS-DRiP amino terminus ($INS\text{-}DRiP_{1-9}$) that binds with high affinity to HLA-DQ8, a class II allele strongly implicated in T1D by genetic studies. Furthermore, it is well known that interferons and other cytokines can induce class II expression in nonimmune cells, including in pancreatic beta cells[11], which suggests that $CD4^+$ cells can interact directly with beta cells presenting $INS\text{-}DRiP_{1-9}$ on class II molecules.

The authors' story now takes a surprising twist: $INS\text{-}DRiP_{1-9}$ also binds with high affinity to HLA-A2, a MHC class I allomorph prevalent in patients with T1D. Sure enough, $CD8^+$ T cells from patients with HLA-A2-positive T1D recognize $INS\text{-}DRiP_{1-9}$-peptide pulsed

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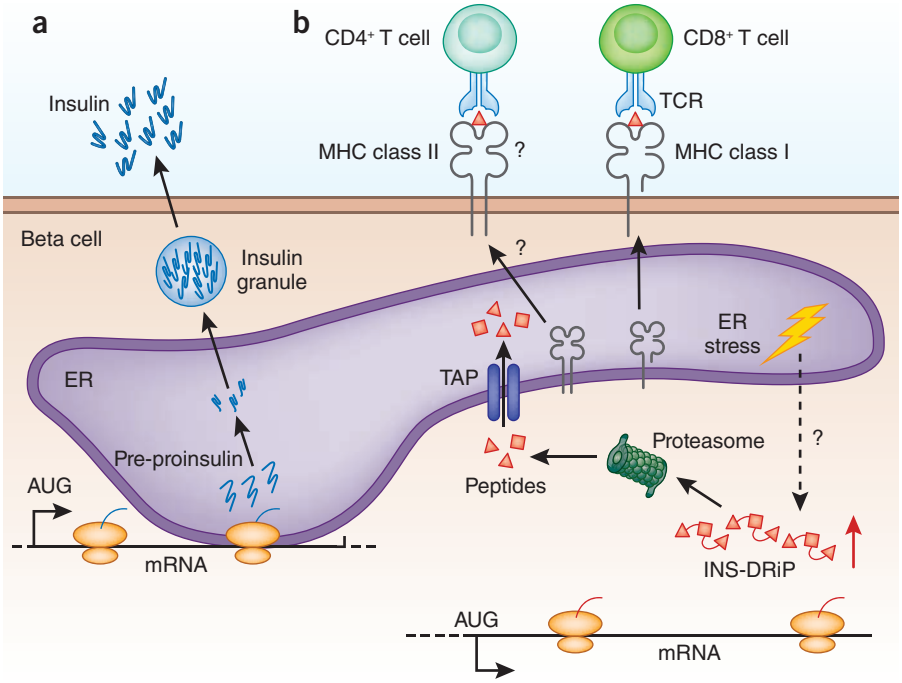

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Figure 1 Insulin-defective ribosomal products (DRiPs) contribute to both CD4$^+$ and CD8$^+$ T cell autoimmunity in type 1 diabetes. (**a**) Canonical translation by ribosomes on the endoplasmic reticulum (ER) generates pre-proinsulin, which is processed into insulin and targeted to specialized granules that release it into the circulation when blood glucose levels increase. (**b**) Kracht *et al.*[1] reveal that insulin mRNA, highly abundant in beta cells, is also translated into a +2 alternative-reading-frame-encoded polypeptide that lacks a stop codon (INS-DRiP) (red). INS-DRiP is predicted to be translated in the cytosol and degraded by proteasomes into peptides that are transported into the ER by TAP (a transporter associated with antigen processing), where it is then loaded onto MHC class I molecules for immunosurveillance. A peptide formed by the nine NH_2-terminal residues from INS-DRiP binds to both MHC class I and class II molecules and is recognized in each case, respectively, by CD8$^+$ and CD4$^+$ T cells, although class II presentation of INS-DRiP by beta cells remains to be established. ER stress seems to favor the synthesis of INS-DRiP, and might trigger its autoimmune recognition.

cells, and critically, kill isolated pancreatic beta cells that express HLA-A2, a finding that is consistent with the generation of INS-DRiP$_{1-9}$ from DRiPs encoded by insulin mRNA. Such T cells are present in patients with recent onset or long-term T1D, consistent with their long-term participation in islet destruction.

On the basis of these findings, Kracht *et al.*[1] propose that an aberrant translation product, whose synthesis is enhanced by ER stress, generates a neoantigenic peptide binding to both class II and I molecules that activates helper and killer T cells to accelerate autoimmunity in type 1 diabetes.

It will be important to establish in future studies how INS-DRiP truly arises in beta cells. Kracht *et al.*[1] find no evidence for alternative splicing of insulin mRNA that would generate an mRNA with a standard open reading frame encoding INS-DRiP. However, further studies are needed to confirm the proposed downstream +2 initiation and to determine how cells clear translationally frozen ribosomes in the absence of a stop codon, as well as to understand the consequences of translating the poly-A tail as poly-Lys. Furthermore, what is the significance of the diabetes-associated genetic polymorphism identified by Kracht *et al.*[1] in the untranslated region of the normal insulin reading frame that results in a two-amino-acid substitution in INS-DRiP? Does it influence the level of INS-DRiP presentation on class I or class II molecules? Given that this sequence is removed from the antigenic peptide, it is unlikely to affect protease liberation of the peptide. Therefore, does it influence the metabolic stability of INS-DRiP, or somehow modulate its entry into antigen-processing pathways by another mechanism? It also remains to be established whether and how INS-DRiP$_{1-9}$ is loaded onto MHC class II molecules in beta cells. Is the endogenous route of peptide loading used[12], and if so, how common is this route, as compared to classical loading in endocytic compartments, in the generation of class II–associated peptides in autoimmunity and cancer?

A key question for understanding T1D is to investigate the nature of the initial proinflammatory insult that sets the stage for INS-DRiP participation in pathogenesis, and whether inflammation-induced mistranslation is required for pathogenesis. Furthermore, is the dual recognition of a single mistranslated peptide by both CD4$^+$ and CD8$^+$ T cells simply a remarkable coincidence, or a key feature of the autoimmune process? The latter case would suggest that autoimmune DRiP-target antigens are so infrequent that each antigen has to break both CD4 and CD8 tolerance. This raises the general questions of how common noncanonical translation is *in vivo*, and how to find a balance between the synthesis of physiologically relevant peptides and proteins and the production of potentially dangerous polypeptidic junk that must be destroyed as quickly as possible. Does this ratio change once disease-associated pathological changes begin? Finally, what can be done to reduce the synthesis of pathological DRiPs or minimize their impact on cellular physiology and autoimmunity, as with INS-DRiP, in T1D[1]?

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The authors declare no competing financial interests.

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