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Unravelling beta cell destruction in type 1 diabetes

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CHAPTER 8

Scientific summary

Type 1 Diabetes (T1D) is the consequence of the progressive destruction of the pancreatic beta cells by autoreactive CD8⁺ T cells, resulting in insulin deficiency and impaired blood glucose homeostasis. Risk of developing T1D has been associated with genetic factors, however genetic susceptibility is not sufficient to drive disease pathology. Endoplasmic reticulum (ER) stress in dysfunctional beta cells caused by environmental triggers is likely involved in breaking peripheral tolerance and drive disease pathogenesis. The ER is essential for insulin production and secretion by beta cells. Perturbations of the ER and the accumulation of unfolded proteins, lead to activation of the unfolded protein response. Signaling cascades induced by the activated ER sensors PERK, ATF6, and IRE1 α attempt to reduce the ER load and increase the ER capacity in order to restore homeostasis. In this thesis, the effect of the inflammatory microenvironment on human beta cell function and immunogenicity has been investigated.

Insulin is believed to be a primary autoantigen involved in beta cell destruction in T1D patients. The signal peptide of preproinsulin (PPI) is a major target of CD8⁺ T cells. Generation and presentation of this epitope, SP_{15-24'}, is mediated via signal peptide peptidase and ER aminopeptidase 1 (ERAP1). In **chapter 2**, we demonstrated that ER stress induced by proinflammatory cytokines facilitates the processing of SP_{15-24'} via upregulation of ERAP1. This upregulation was mediated by a decrease in miR-17 via IRE1 α RNase activity. MiR-17 overexpression or IRE1 α inhibition blunted the effect induced by proinflammatory cytokines. These data provide direct evidence of the implication of ER stress in autoantigen processing and beta cell immunogenicity.

T1D was previously assumed to be caused by impaired thymic T cell education. However, the implication of beta cell dysfunction prior to disease onset rather supports an alternative explanation where the beta cells actively contribute to their demise. Besides invading pathogens, the immune system is also trained to eliminate dysfunctional cells to prevent them from becoming harmful for the entire organism. A well-known example of this is an effective antitumor response. In **chapter 3.1** we compared the tumor microenvironment with the microenvironment of islets during insulinitis and argue that diabetes might be result of successful clearance of dysfunctional cells by the immune system. Neoepitopes are important T cell targets in an effective anti-tumor response as they discriminate functional-self from dysfunctional-self. Cancerous cells have been shown to generate a wide variety of neoepitopes resulting from

mutations, splice variants, defective ribosomal products, and posttranslational modification (PTM) of native proteins. In **chapter 3.2** the contribution of post-transcriptional and post-translational modifications in the generation of beta cell neoantigens is evaluated. Compelling evidence supports the role of environmental stress-induced neoepitope formation in T1D pathogenesis. Altogether, these examples suggest that beta cell destruction resembles an effective anti-tumor response. A better understanding of the processes and the origin of neoepitopes is essential in our battle against T1D.

Based on this hypothesis, in **chapter 4** we investigated insulin as source of neoepitopes generated by unconventional translational processes. The high translation rate of insulin combined with the intrinsic physiological production pressure during metabolic needs, made this an interesting candidate. This led to the identification of an alternative open reading frame within insulin mRNA. Production of this defective ribosomal product (DRiP) might be associated with ER stress, as was observed in surrogate beta cells. Interestingly the resulting polypeptide contains an immunodominant N-terminus that can be presented in HLA class I and class II. CD8⁺ T cells directed to this epitope were found in a higher frequency in the circulation of patients. Furthermore, these CD8⁺ T cells killed human pancreatic beta cells *in vitro* which was enhanced during inflammatory conditions, underscoring its relevance to disease pathogenesis.

Chapters 2, 3 and 4 emphasize the importance of ER stress in the processing and generation of beta cell (neo-)epitopes and autoimmunity. Therefore, the ability to monitor ER stress is essential in understanding disease pathogenesis and might contribute to the development of therapeutics. In **chapter 5**, we designed a bioluminescent reporter assay for monitoring ER stress in human beta cells. This reporter construct encodes a XBP1-Gussia luciferase fusion protein. Endogenous XBP1 is unconventionally spliced by IRE1 α upon ER stress, resulting in the shift of the reading frame and therefore translation of an elongated C-terminal protein. Similarly, in this fusion construct, the C-terminal Gussia Luciferase is only in the correct reading frame after IRE1 α -mediated splicing, resulting in stress-induced Luciferase expression. ER stress quantification was accurate when compared to classical ER stress quantification methods and less laborious in execution. In addition, reporter expression driven by the human insulin promotor restricts the reporter to beta cells specifically. Therefore this assay allows specific monitoring of the ER stress status of beta cells in isolated human pancreatic islets. We envisage that this reporter can be

used in studies on the origin of ER stress, as well as a drug screening platform to reduce ER stress.

Insulin-production by the beta cells is of vital importance, paradoxically, as stated earlier, insulin remains also a main autoantigen in T1D. Besides insulin and INS-DRiP, we discovered an alternative insulin gene-derived splice variant (INS-splice) that contains the PPI signal peptide, the B-chain and the C-terminus of INS-DRiP. In **chapter 6**, we analysed the expression of these insulin variants in human pancreatic islets using specific custom-made antibodies. In accordance with our previous results, the N-terminus of INS-DRiP containing the CD8⁺ T cell epitope, was detected in pancreatic beta cells. Yet, the C-terminus of INS-DRiP was not detected, implying that the polypeptide does not exist as mature polypeptide, maybe as consequence of rapid nonSTOP protein decay. The mRNA encoding INS-splice is present in beta and delta cells, however, the polypeptide appears restricted to delta cells. More specifically, the INS-splice polypeptide was localized in the delta cell granules. We suspect this might be the consequence of the presence of the PPI signal peptide in INS-splice, which drives the co- and posttranslational processing. Although the function of INS-splice remains elusive, we envision it might take part in beta and delta cell lineage development, while it is conceivable that it contributes to maintaining mature endocrine cell plasticity as alternative splicing is an important mechanism in lineage development and maintaining pluripotency. Its localization in delta cell granules also suggest a potential paracrine or endocrine function.

ER stress in beta cells seems to drive T1D etiology by attracting immune cells, increased autoantigen processing, and neoepitope generation. Neoepitope formation might have a crucial role in the loss of peripheral tolerance and beta cell destruction. Therefore identification of these neoepitopes is important for monitoring disease progression, as well as for the development of immunotherapies in order to re-establish peripheral beta cell tolerance. In addition, our results point to the importance of research to the causes of beta cell dysfunction and propose restoration ER homeostasis as potential therapeutic target.

ADDENDUM

Nederlandse samenvatting

Curriculum Vitae

List of publications

Dankwoord

