

Editorial

REGARDING THE TRIGGER OF THE BETA CELL AUTOIMMUNITY

Cristian Guja^{1,2,✉}, Constantin Ionescu-Tîrgoviște^{1,2}

¹ National Institute of Diabetes, Nutrition and Metabolic Diseases “N. C. Paulescu”, Bucharest

² University of Medicine and Pharmacy “Carol Davila”, Bucharest

The final aim of diabetology, prevention of diabetes (in this discussion prevention of the autoimmune type 1 diabetes – T1D), finds us unprepared. This is because prevention cannot be done without a certain prediction while T1D prediction cannot be done without a good understanding of its pathogenesis. The main obstacle was represented by the limited (and only occasional) access to the study of the pancreas morphology, usually only once in a patient sometimes with an uncertain clinical phenotype, i.e. not very specific for T1D. The studies of the anti-β-cell autoimmunity performed on some animal models were hastily extrapolated to the pathogenesis of human. These pathogenic models represented the basis for the human testing of some preventive methods. The first were performed in the 1980's with cyclosporine and were followed by newer generations of non-specific immune-suppressors, usually without conclusive results [1,2]. Being applied at the clinical onset of diabetes, these methods could not have a preventive nature but represented rather a treatment.

After the genetic revolution represented by the Genome Wide Studies, it was hoped that a genetic risk score will allow for a better prediction of the disease. Some progresses were done but identifying subjects at increased risk for T1D only from the “first degree relatives” of

T1D patients is not efficient since >80% of T1D patients represent isolated cases and will not be identified by such studies. The detection of subjects with increased risk for T1D based on the identification of some highly diabetogenic genotypes in the presence of at least 2 anti-beta cell antibodies in significant titers [3] will select patients with already present autoimmunity, which usually have already passed beyond the point of “no return”.

Realizing the dead-end reached by the studies analyzing the pathogenesis of T1D (mainly because of the lack of proper identification of the anti-beta cell autoimmunity trigger), Størling et al. recently published an interesting hypothesis on this topic [4]. Thus, members of the research group that established in 1974 the “immune-genetic” theory of T1D [5,6] launched “for debate” a new hypothesis that was discussed during the past 10 years as possible pathogenic mechanism for other autoimmune diseases [7]. This hypothesis is based on the role of some antigenic “neo-epitopes” derived from the post-translational changes of some proteins expressed inside the beta cells. The Danish group considers for T1D that “*a classical autoimmune disease should be reconsidered since the immune response may not be directed against natural beta cell proteins*”. We notice that the notion of autoimmunity

✉ Managing Editor Rom J Diabetes Nutr Metab Dis
5-7 Ion Movila Street, Bucharest 2, Romania; Tel: 004 021 210.84.99; Fax: 004 021 210.22.95
corresponding author e-mail: cristian.guja@b.astral.ro

“trigger” was replaced with that of “*initiation phase*” which would be independent of beta cell antigens and lymphocytes but dependent of the cytokines produced following the intervention of some non-identified environmental factors, possibly represented by some viral infections.

To our best knowledge, such factors, intensively studied during the last 4 decades were never precisely identified [8]. This is one of the reasons for which during the last years we analyzed the possibility that a pathogenic factor could be hidden inside the pancreatic beta cell itself [9-12]. In fact, the Danish group of Jørn Nerup [4] searched the cause of T1D also inside the beta cell, starting from another hypothesis that obviously requires some objective arguments and, if possible, confirmation. In fact, in the same issue of *Diabetologia*, Ake Lernmark, another great figure of T1D research, questioned this hypothesis stating that: “*Data on PTM (post-translational modification) of islet autoantigens are scarce and readers should not believe that the PTM hypothesis is supported by strong experimental evidence*” [13].

While the Danish group invoked the possibility of the antigenic *neo-epitopes* genesis following some post-translational changes of some intra beta cell proteins, we have already for a long time sustained that the post-translational defect could be represented by the incomplete processing of proinsulin inside the Endoplasmic Reticulum (ER). This defect could be explained by the ER stress induced by the functional overload of these intra-cell organelles whose role is to block the passage towards the Golgi apparatus of the incompletely processed molecules. Any defect in the system of “quality control:” of these molecules, especially in the presence of secretory overload, will lead to the passage in the Golgi Apparatus (GA) of a high number of incompletely processed proinsulin molecules that will appear as such inside the

final secretory vesicles where their concentration will increase. We consider that this excessive increase of proinsulin inside the immature secretory vesicles could represent the elusive” trigger of the anti beta cell autoimmunity.

The second phase of the anti beta cell autoimmunity hypothesized by Storling et al [4], is designated as the “execution” phase, i.e. of beta cell destruction. The question that arises and to whom we should search for an answer is: why this autoimmune attack targets specifically pancreatic beta cells. We think that this specific attack is explained by the fact that the beta cells that have immature secretory vesicles and, consequently, carry large amounts of proinsulin are perceived by the immune system as “defect” cells, incompatible to performing normally their secretory function. Our hypothesis is based on the antigenicity of the specific beta cell proteins (proinsulin/insulin) when they are found inside the secretory vesicles unable to mature, i.e. to produce an almost complete splitting of proinsulin to insulin and C peptide. This process requires that the intra-vesicular pro-convertases are activated by an acidic (*pH* 5–5.5) environment generated by the proton pump from their membrane, requires the presence of an optimal Zn concentration provided by the Zn transporter (ZnT8) located again in the membrane of the SV. Other conditions are represented by the presence of an optimal level of calcium Ca^{+2} and an optimal ratio between amylin and proamylin [11,12]. These are the “mature” secretory vesicles that appear in electronic microscopy with a dense nucleus represented by insulin deposits in the form of insulin hexamers formed by covalent bonds with 2 Zn atoms, each one binding 3 molecules, in the form of specific hexagonal crystals. In the peripheral trans-lucid zone of the SV are found the PC2 and PC3 pro-convertases and carboxipeptidase H, chaperone molecules and

the other secretory molecules (amylin, etc.). While Orci in 1985 characterized the beta cell as an insulin factory” [14], we think that the beta cell is rather a factory of “completely matured secretory vesicles” [11]. Only such a secretory vesicle will answer promptly and efficiently to its physiologic stimuli [15].

In order for the pancreatic beta cell to produce such tightly packed secretory vesicles [16], it is required that the assembling of the 44 specific beta cell molecules to be found at the right place and to be used at the right time. The recent GWA scans for T1D genes identified approximately 50 genes [17,18]. The majority of these genes encode proteins associated with the innate and acquired immune function [19]. For part of these genes, the functional relevance of the encoded protein is not yet known. They could encode either some putative beta cell proteins or molecules associated with cells involved in the immune response.

From these genes, we mention the *SLC30A8* that encodes the Zn transporter, initially identified in the first wave of GWAs for T2D. This gene, even if associated with T2D, encodes an antigenic protein that is rather studied as a T1D beta cell auto-antigen and not according to its major role in the process of SV maturation. We have some arguments that the two major diabetes phenotypes, T1D and T2D, (unfortunately usually analyzed completely separately according to the “*all white or all black*” theory) represent in fact only the two extremes of a “*continuum*” of clinical forms. For the diabetic syndrome, the balance between the intensity of the immune system defect and of the beta cell secretory defect (an important center of control and command for the energy metabolism severely impacted by the environmental factors)

could explain all the diabetes phenotypes encountered in clinical practice [20].

We think that the innate immunity could react against the immature secretory vesicle inside the pancreatic beta cell (whose function becomes inefficient) initiating a tightly regulated pro-apoptotic autoimmune attack that evolves slowly and progressively.

Since the main characteristic of the “defect” secretory vesicle is their high content of proinsulin, we hypothesized that this could represent the trigger of the anti beta cell autoimmunity. The appearance of the anti-insulin (anti proinsulin) antibodies (the first to appear in the natural history of the autoimmune beta cell destruction) could be explained by the presence of antigenic epitopes on both the proinsulin and insulin molecules.

While some cells are destroyed during the apoptotic process, exposing of the other beta cell auto-antigens will trigger the initiation of subsequent waves of autoimmunity (antigen spreading) targeting the GAD, IA2 and ZnT8 molecules. In the same time, the process will expand to a higher and higher number of islets since the remaining beta cells will take the secretory function of the already destroyed cells, their secretory demand increasing severely. The secretory overload of these remaining cells will increase their vulnerability to the autoimmune attack. Only when the number of destroyed beta cells will reach 90% the clinical signs of diabetes will become obvious. Failure of all preventive measures attempted at this stage is explained by the extremely delayed nature of the intervention, taking into account the limited regeneration potential of the remnant beta cells, especially after the age of 20 years [21].

REFERENCES

1. **Chatenoud L, Bluestone JA.** CD3-specific antibodies: a portal to the treatment of autoimmunity. *Nat Rev Immunol* 7: 622-632, 2007.
2. **Chatenoud L.** Therapeutic targeting of B cells and T cells in autoimmune diabetes: is it a solution? *Diabetes* 62: 2659-2661, 2013.
3. **Mbunwe E, Van der Auwera BJ, Vermeulen I et al.** HLA-A*24 is an independent predictor of 5-year progression to diabetes in autoantibody-positive first-degree relatives of type 1 diabetic patients. *Diabetes* 62: 1345-1350, 2013.
4. **Størling J, Overgaard AJ, Brorsson CA et al.** Do post-translational beta cell protein modifications trigger type 1 diabetes? *Diabetologia* 56: 2347-2354, 2013.
5. **Nerup J, Platz P, Andersen OO et al.** HLA antigens and diabetes mellitus. *Lancet* 2(7885): 864-866, 1974.
6. **Bottazzo GF, Florin-Christensen A, Doniach D.** Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2(7892): 1279-1283, 1974.
7. **Dunne JL, Overbergh L, Purcell AW, Mathieu C.** Posttranslational modifications of proteins in type 1 diabetes: the next step in finding the cure? *Diabetes* 61: 1907-1914, 2012.
8. **Egro FM.** Why is type 1 diabetes increasing? *J Mol Endocrinol* 51: R1-R13, 2013.
9. **Ionescu-Tîrgoviște C, Guja C.** The various phenotypes of diabetes and the endoplasmic reticulum of the beta cell. *Rom J Intern Med* 45: 287-291, 2007.
10. **Ionescu-Tîrgoviste C, Guja C.** Proinsulin, proamylin and the beta cell endoplasmic reticulum: The key for the pathogenesis of different diabetes phenotypes. *Proc Rom Acad, Series B*, 2:113-139, 2007.
11. **Ionescu-Tîrgoviște C.** Proinsulin as the possible key in the pathogenesis of type 1 diabetes. *Acta Endocrinologica* 5: 233-249, 2009.
12. **Ionescu-Tîrgoviște C, Guja C, Cristescu V, Guțu D.** The role of the pancreatic amyloid in the pathogenesis of type 2 diabetes. *Proc Rom Acad, Series B* 1: 21-34, 2010.
13. **Lernmark A.** Is there evidence for post-translational modifications of beta cells autoantigens in the aetiology and pathogenesis of type 1 diabetes? *Diabetologia* 56: 2355-2358, 2013.
14. **Orci L.** The insulin factory: a tour of the plant surroundings and a visit to the assembly line. The Minkowski lecture 1973 revisited. *Diabetologia* 28: 528-546, 1985.
15. **Michael DJ, Xiong W, Geng X, Drain P, Chow RH.** Human insulin vesicle dynamics during pulsatile secretion. *Diabetes* 56: 1277-1288, 2007.
16. **Rorsman P, Renström E.** Insulin granule dynamics in pancreatic β cells. *Diabetologia* 46: 1029-1045, 2003.
17. **Stefan M, Zhang W, Concepcion E, Yi Z, Tomer Y.** DNA methylation profiles in type 1 diabetes twins point to strong epigenetic effects on etiology. *J Autoimmun* doi: 10.1016/j.jaut.2013.10.001, 2013. [Epub ahead of print]
18. **Erlich HA, Valdes AM, Noble JA.** Prediction of type 1 diabetes. *Diabetes* 62: 1020-1021, 2013.
19. **Todd JA.** Etiology of type 1 diabetes. *Immunity* 32: 457-467, 2010.
20. **Ionescu-Tîrgoviste C, Gagniuc P, Guja C.** The promoters of genes associated with type 1 and type 2 diabetes seem to have some specific features. *Diabetologia* 55[Suppl1]: S123, 2012. (Abstract 280)
21. **Cnop M, Hughes SJ, Igoillo-Esteve M et al.** The long lifespan and low turnover of human islet beta cells estimated by mathematical modeling of lipofuscin accumulation. *Diabetologia* 53: 321-330, 2010.