WHAT IS BEFORE THE AUTOIMMUNE SEROCONVERSION IN TYPE 1 DIABETES?

C. Ionescu-Tîrgoviște

“N.C. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases, Clinic of Diabetes, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Abstract

In the past years a high interest has been observed for understanding the early stages of type 1 diabetes. That interest has been stimulated by the failures of the various “preventive” approaches of the autoimmune mechanism operating in this phenotype, carried out in young diabetic patients, soon (several months) after the clinical onset of the disease. Unfortunately, the recent Statement of three scientific organisations from the USA proposed a reconsideration of the well-known classical stages, not going backward to know better the true early onset of the autoimmunity, but refining only the second part of the classical stages which are closer to the clinical onset of diabetes (when the β-cell mass/function is about 70% already irreversibly lost). In opposition with the above mentioned initiative, our effort has been devoted to the detection of earlier stages of diabetes which silently operate before the detection of the first islet autoantibodies (mainly proinsulin/insulin antibodies) which strangely was omitted in the new mentioned reclassification of preclinical stages of type 1 diabetes.

Key words: type 1 diabetes, autoimmunity, prediction, prevention.

INTRODUCTION

For more than a decade I sustained on various occasions (1-8) that the definition of diabetes using as a unique criterion the “epiphenomenon” hyperglycemia, and not the “phenomenon” which is the loss of the β-cell mass/function, is a long standing error. This change must be common for any phenotype of diabetes, which are more than the two classical type 1 and type 2 diabetes that had been wonderfully described in 1883 by the French clinician Etienne Lancereaux (1829-1901) under the name of “slim” (or pancreatic) diabetes and “fat” (or constitutional) diabetes, respectively (9). I argued that it is less important than in autoimmune type 1 diabetes (the former “juvenile” diabetes) the loss of β-cell mass/function is rapid, and in type 2 diabetes (the former “maturity-onset” diabetes) is slow or very slow, than the fact that in all these distinct phenotypes, the causal phenomenon is the same: a decrease in the β-cell mass/function, even if the mechanism can be different.

In their recent Statement (11), Juvenile Diabetes Research Foundation (JDRF), Endocrine Society (ES) and American Diabetes Association (ADA) have proposed the reclassification of the stages of the natural history of type 1 diabetes dating from 1986 (10). The aim of adopting this classification was that to promote “precision medicine involving the tailoring of optimal therapy to specific individuals at specific stages of the disease” (11). I agree with this important aim, but we all know that today, even if we have a 95% prediction for an early decompensation of blood glucose regulation (hyperglycemia), we can only illegally use the diagnosis of type 1 diabetes (respectively, type 2 diabetes), according with the actual regulations. The currently used principles put the cart before the horse, whereas we all must fight to adopt the idea that it is better to put the cart behind the horse. Moreover, in our view, to wait for total decompensation of blood glucose regulation (hyperglycemia) can be expressed as the popular saying: “to close the stable after the horse has already been stolen”. If after adoption of the new classification, the diagnosis of type 1 diabetes will be accepted also for the prehyperglycemic stages of diabetes (one of my dreams for many years), then the interest for earlier detection of this dangerous disease - which is really present and destructive in a shorter or longer period of a continuous loss of beta-cells, will be stimulated.

I agree with the above mentioned Statement (11) in dividing the former stage 3 of Eisenbarth’s classification (10) in two sub-stages: Autoimmunity + Normoglycemia/ Presymptomatic Type 1 Diabetes (stage 1); Autoimmunity + Dysglycemia / Presymptomatic Type 1 Diabetes (stage 2). The first was
clearly based mainly on the data of Ziegler et al. (12), and the second by the data published by Krischer et al. (13). Both are presymptomatic, and only in the last one (stage 3 Autoimmunity + Dysglycemia/Symptomatic) the symptoms appear. Beside this classification, the genetic susceptibility and genetic risk of T1D has been placed as a pre-stage 1.

What critical remark can be made to this obvious “in hurry” Statement?

First, the authors use the term “dysglycemia” in two different stages, the last one (stage 3) being a full, and sometimes severe, metabolic decompensation, not rarely associated with ketosis or even with ketoacidosis. It is known that the term dysglycemia is currently used to designate a prediabetic stage mainly in type 2 diabetes (T2D). In my opinion, to use it in the stage 3 of the proposed classification should be reconsidered.

Second, in this classification there are not any remarks about anatomic and histological data (the possible relationship of the three stages with insulitis, the main clear and specific lesion in T1D). We know that insulitis has been described in young diabetic patients, dead soon after the onset of diabetes (in Stage 3 of the Statement), but its presence can be sustained by testing some plasma chemokines, cytokines, microRNAs and in the next future, hopefully imagistic methods (14).

Third, few is mentioned about the huge heterogeneity of various phenotypes of diabetes, with the special mention to the autoimmune diabetes in children. This phenotype (called also “pediatric diabetes”) can appear in the first year of life, but also between 1-3 years, between 3-10 years, or between 11 and 16/18 years. Age at onset of diabetes is an extremely important parameter, because various sub-groups show several genetic, clinical and, probably, pathogenetic particularities. In line with this aspect, few specific references have been made on the Intermediary Diabetes Mellitus (IDM), as we proposed to be named the type 1 diabetic patients with onset between 18 and 40 years of age (15, 16), the former LADA (Latent Autoimmune Diabetes in Adults). That represent a high percentage (about 15%) of type 1 diabetic patients. Nothing has been mentioned about the onset of the type 1 diabetes in higher ages in the fifth, sixth or higher decades of life (15, 21). Is the pathogenic mechanism the same in all these phenotypes? The answer to this question is important, because the prevention of a disease must be preceded by its prediction (by specific markers) and prediction cannot be conceived without the knowledge of the disease pathogenesis. It will become obvious that the proposed new classifications are not appropriate for the prevention of type 1 diabetes, as it results from the “completion” studies presented in their Table 2, but whose final results were disappointing. The development of the monoclonal antibodies therapy that target both the T cells and B cells has been used with the hope that it will prevent the further destruction of the few remaining beta cells in the patients with new onset type 1 diabetes. Our prediction is that any attempt to prevent diabetes when the intervention is made, only in the stage 3 of the Statement, but also in the stage 2 or even stage 1, will fail. This could be a pessimistic view, but we consider that intervention must be done before entering the stage 1 of the new classification.

The forth remark is that the authors did not discuss anything about the biological reaction that is involved in the first conflict between the pancreatic β-cells and the immune system. Indeed, this true war between the pancreatic beta cells and the immune system takes place without any apparent symptom even before the first seroconversion appears. However, the strong reaction of the two parties in this conflict is associated to release of several cytokines, chemokines, RNAs or other stress molecules, which can be used as early markers of the assault of the various components of the huge immune system on the pancreatic β-cell (7). No reference was made to the real steps (in our view two or three), which precede the 1st stage in the new proposed classification. In reality, I believe that this “early stage” of diabetes is not so early as the authors claim.

The fifth remark refers to the disappearance in the new classification the “Trigger” (Precipitating event) placed in the Einsenbart’s classification between “Genetic predisposition” and the “Overt immunologic abnormalities”(10). For three decades, the trigger of the autoimmune process has been associated with several environmental factors, mainly various viral/microbial agents, nutritional factors or maternal/neonatal events (17-21). However, the causal relations between such factors including viral one and T1D is not yet fully clarified (6, 7, 15). In my view, any infection, including the migration of some viral/germs from the huge gut microbiome can sometimes act as a trigger of anti-beta cell autoimmunity. More often such agents can it can invade the pancreatic islets after the onset of diabetes, amplifying the insulitic process.

Among the environmental factors, I want to underline the importance of the so-called “endocrine-disrupting chemicals” which were recently approached in the light of overall medical costs related to their
Early autoimmune diabetes

The predisposition to diabetes

If we have a look on the predisposition to autoimmune diabetes from the genetic point of view, also analysed by Insel et al. (11), there are mentioned some various genetic risk scores proposed by several groups of researchers, generally having a low prediction over the current clinical risk scores (15, 22).

In my view, the selection of “at risk” subjects will be easier and with a good cost/benefit ratio if it is based on the prediction for developing diabetes in those individuals who do not carry the protective genes, which are only few. If these subjects have not diabetes in their families, they can be excluded from the various trials proposed for prevention of type 1 diabetes.

If we look to the non-HLA gene associated with T1D, the insulin gene (INS), in fact encoding the pre-proinsulin molecule, carries the antigenic epitopes on all its three important segments: signal peptide, proinsulin and insulin (3, 5). This explains the presence of proinsulin/insulin autoantibodies which are, at least at young ages, the first, or among the first antibodies, that appear in the circulation. This strongly suggests a possible role of these molecules in the pathogenesis of T1D.

The second important gene is Zn-T8 (SLC30A8) encoding the isoform 8 of the zinc transporters (23, 24, 25) which has been initially included to the genes associated with T2D, but the antibodies against this antigenic molecules show its relationship with T1D. The same significance has the gene TCF7L2 encoding a complex molecule influencing many β-cell biochemical pathways (the development of pancreatic islets among others), and other extra β-cellular pathways related to the several changes in the huge system controlling the metabolism of the human body (26). In fact, these three genes, among others, may suggest the unitary character of the diabetic syndrome (5).

A new approach of genomic studies

In one of our new post-genomic approaches (27), we have advanced the hypothesis that what is important in the transcription of a gene is the structure of their promoters, the region of ~500 nucleotides preceding the starting point of a gene. Because the gene transcription depends on the transcription factors acting through promoters, the level of transcription could be higher or lower (28-31). Our new cryptographic method which analyses, nucleotide by nucleotide, the content of promoters in C+G % versus Kappa Index of Coincidence (KIC) generates a specific pattern which is specific for T1D, IDM or T2D, as can be seen in Figure 1 (27-29).

It results that Intermediary Diabetes Mellitus (IDM) is a term which corresponds with the reality

---

**Figure 1.** The distribution of promoters of genes associated with type 1 diabetes, type 2 diabetes and IDM (27).
of a continuum in the diabetes phenotypes, with IDM standing between T1D and T2D, but with some overlap between them. The term IDM may be a more appropriate one than the “strange” LADA (Latent Autoimmune Diabetes in Adults) which has no coherent definition as we mentioned in one of our recent papers (21, 27, 28).

**Which is the culprit in T1D: the immune system or the pancreatic β-cell?**

For more than a decade, we (5-8) advanced the hypothesis that the predisposition to diabetes includes two different defects: one is the immune system, indicated by the islets autoantibodies, and the other one inside the β-cell. Before their appearance, a subtle and silent war is developing against those pancreatic islets containing β-cells unable to produce mature secretory vesicles, and even more specific the extracellular matrix around the β-cell. Our view, which has been presented on several occasions (5-7), stresses the outstanding importance of the extracellular/ intercellular matrix between two β-cells, between the β-cell and the endothelial cell, and between β-cell and the other secretory cells in the islets. The elaborate construction of such complex network of molecules between these cells results from the cooperation between β-cells with other proxy cells (33-36).

As β-cells carry a genetic defect in one or more biochemical pathways (30), taking part in the maturation of the secretory vesicles (the main function of this cell), they are also unable to take part in producing a surrounding strong protective barrier. Moreover, the chemokines produced by such cells can be detected by the immune system (mainly dendritic cells), as a “danger” signal. A hyper-reactive immune system will test the functionality of these cells, and if their defect in important enough, a T cytotoxic clone specifically directed against the β-cell antigens will be produced in the numerous peri-pancreatic lymph nodes. That time, the immune B cells will produce the antibodies which can be detected in the circulation and used as a marker one of the early stages of type 1 diabetes (30). Applying immune therapy later than this stage, will not be able to halt the ongoing immune process as resulted from the several studies carried out in the last years (37- 41).

**Instead of conclusion: where will we go from here?**

What has been lately remarked in diabetes research is that the majority of data regarding type 1 diabetes is obtained from animal models (6, 7, 42, 43) like non-obese diabetes (NOD) mice or bio-breeding (BB) rats. This is why Krogvold et al. took a new courageous step in analyzing the tail of the human pancreas (43). The first results of the n-POD (network of Pancreas Organ Donors) have been published recently. One of them is related to the investigation of viral presence in the pancreas (44), and the other is related to the viability of the β-cells in the young adults (24-35 years) recently discovered with type 1 diabetes (18).

As I mentioned in one of my reviews (7), in this study, they demonstrated that after the islet and β-cells isolation it is possible to partially regain the insulin secretion capability after the placement of these cells in the normal glycemic conditions. However, this information did not have a great importance because the β-cell remaining inside the pancreas will have the same fate of declining progressively the remaining β-cells, as it is proved by the decreasing C-peptide levels (13).

Because information obtained from the animal models regarding the reversibility of the insulitis and diabetes was not useful for humans when they were transferred using various immune modulatory approaches, a need for extending the studies on human pancreas becomes an obvious urgency. In order to have a map of the islets in the normal human individual, we recently published the first careful study of the architecture of islets, including their dimension, density and distribution in the head, body and tail (45). As can be seen in Figure 2, the density of the islets in the pancreas is not anarchical, there are two main routes suggesting that the progression of the pathogenic processes (insulitis) might follow a quite specific pattern. It would be of great interest to see if there is a correspondence when the tail of pancreas obtained by Krogvold et al. (43) is superimposed on the islet

**Figure 2.** A 3D representation of heat map showing the density of islets in the head, neck, body and tail of a normal pancreas. Two routes starting from the body to the tail can be seen (45).
architecture we observed in this region for the pancreas we analysed.

The socio-economic globalization had its good and its less good consequences but, for sure, the globalization of scientific community could amplify the effort for the prevention of diabetes. For such a prevention, we need a good prediction and for a good prediction, we need to know the first diabetogenic movements which in our view are much more than that presented in the statement recently published. The second part of the pathogenic mechanism is quite well defined by the authors of this consensus but, unfortunately, they are too late for halting the destruction of β-cells. I propose as a research target a better understanding of what happens inside the “black-box” of pancreatic islets, where the pathogenic movements are initially silent, but very active and passing through many stages afterwards, in which prodiabetogenic forces have to confront with the protective ones, explaining why this phenotype of diabetes is really a remitting/relapsing phenomenon (13). In order to do this we can use new bioinformatic methods of analysis that are already in development (27-30, 45, 46). Till then, we will try to conceive a new classification including all the steps which we believe to precede the classification of Insel et al. (11) that was produced mainly to give some support to ongoing trials using the immune modulatory agents.

Conflict of interest
I declare no conflict of interests.

Acknowledgement
This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0429.

References