A SHORT PERSONAL VIEW ON THE PATHOGENESIS OF DIABETES MELLITUS

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Received December 27, 2010

The paradox of diabetes is that, despite the wide array of diabetic phenotypes, it retains a unitary character in that they all result from disturbances in the organism's energy metabolism. As the "classic" definition of diabetes retains decompensation of blood sugar regulation (hyperglycemia) as sole criteria of diagnosis, this could be one of the main arguments for the unitary character of diabetes. The second argument is that a decompensation of blood sugar regulation implies as a *sine qua non* condition a drastic decrease in β-cell mass/function (to less than 50%).
In order to explain the two main phenotypes (T1DM and T2DM), we consider that the rate of β-cellular mass decrease is less important than the loss of β-cell mass in and of itself, which in type 1 is very rapid due to the autoimmune destruction, whereas in type 2 it is slower, due to the high β-cell mass function reserve, which takes years in order for it to become clinically apparent.
That is why we consider that we urgently need a change not only in the definition of diabetes, but also with respect to the criteria of diagnosis, as well as finding a parameter indicating the level of β-cell mass function, in order to split the evolution of diabetes into a prehyperglycemic stage (a decrease in β-cell mass function with normal blood sugar levels) and a late and irreversible hyperglycemic stage, which appears when more that 50% of the β-cell mass is irreversibly lost.

Key words: diabetes mellitus, pancreatic β-cell, T1DM, T2DM.

1. Diabetes is a complex disorder of the energy metabolism, affecting proteins, lipids and carbohydrates altogether[1].

2. Energy metabolism is controlled by multiple biochemical and hormonal influences, conditioned by important genetic and environmental factors[2]. The diabetes syndrome, therefore, includes a myriad of clinical forms, grouped in several more or less specific phenotypes present in the current classification of diabetes.

3. Diabetes can appear from the first day of life until the last year of a long-lived person, the incidence increasing beginning with childhood and reaching a peak around 65 years of age, afterwards declining due to increased mortality from various other diseases after this age[3].

4. The „brain" of the energy metabolism control is the pancreatic β cell in the context of the Langerhans islets, whose secretion can be modulated by other endocrine factors (glucocorticoids, sex hormones, pituitary hormones etc.) and by metabolic influences. The effectory organs/ btissues/cells through which insulin exerts its action are: the *liver* (hepatocytes), with its main function of processing the fuels coming from the intestine after food intake; the *muscles* (myocytes), the main energy consumer; and the *adipose tissue* (adipocytes), which have as main role the storage of non-burned fuels as triglycerides inside the adipocytes.

5. The normal pancreatic beta cell secretes two hormones: insulin and amylin, the C peptide has also some physiological effects which must be taken into account despite the absence of the specific receptors for it. The former (insulin) has been better studied than the latter (amylin). There is data showing the role of insulin in maintaining the pancreatic beta cell function itself, similar with its effect on other insulin-dependent cells/tissues: adipocyte, hepatocyte, miocyte, endothelial cells, and others. Among these others, we highlight the

function of hypothalamic neurons with important role in the regulation of food intake.

6. The β cell secretory defect is the fundamental defect, identifiable in every phenotype of the incommensurate diabetes syndrome. This is proved by the numerous studies that identified discrete secretory β cell defects in first degree relatives of diabetic patients. This defect precedes the alteration of blood glucose regulation (hyperglycemia) with years or decades. In our view, the most important indicator of this defect is represented by the increase of proinsulin inside the beta cells and in systemic circulation. This defect has been found by us in both T1DM and T2DM[4–7]. It has also been found by several other authors in the offspring of both T1DM and T2DM patients[5–7].

7. Recent genetic studies confirmed the presence of some molecular defects involved in the secretory function that condition the increase in proinsulin and decrease in mature insulin, both inside the β cell and peripheral circulation[8, 9].

8. The amylin defect (amyloidal transformation of amylin) seems to be secondary to the proinsulin/insulin defect but the exact trigger of this transformation was not yet fully understood[10–13].

9. If we accept that the beta cell secretory defect is the fundamental disorder in diabetes mellitus, it should be stated that this defect refers to the main function of these cells. This function is not only to secrete insulin, but mainly to produce mature secretory vesicles (SV). The pancreatic beta cell is not an „insulin factory"[14, 15], but rather a "*secretory vesicle factory*"[16, 13].

10. „Mature" secretory vesicles contain ~200.000 insulin molecules (produced by the enzymatic split of proinsulin into mature insulin a C peptide) deposited in the center of the vesicle as hexameric crystals with two Zn atoms in an acidic (pH 5–5,5) intra-vesicular milieu. In the peripheral halo of the secretory vesicle remain in soluble form molecules of amylin, C peptide, enzymes, chaperones and many ions that, together with water, form a colloidal complex[13].

11. An „immature" secretory vesicle contains only partially cleaved proinsulin and pro-amylin. This is why the intensely electron dense core (characteristic for the mature vesicle) is missing and the pH doesn't reach the very low figures reported above. It is probable that some components of the exocitotic molecular apparatus present on the surface of the immature secretory vesicle membrane will not fit with the corresponding docking components from the inner layer of the cell membrane. This could explain the „discrete" insulin secretion disturbances that appear in the pre-hyperglycemic stages of diabetes: disappearance of the physiologic insulin secretion pulses (with frequency of ~12 min, ~140 min and 24 hours); decrease or disappearance of the first phase insulin response (5–7 min); the delay between the increase in blood glucose and increase in plasma insulin levels (the final cause of hyperglycemia). Subsequently the progressive decrease of the insulin response will be recorded (in T2DM this is never completely abolished).

12. The amyloidal transformation of amylin might be present only in the immature vesicles as a consequence of this immaturity that affects the processing of proinsulin/pro-amylin or the quantitative disequilibrium between these two components. The C peptide could also take part in this process.

13. The cause of the decreased capacity of the beta cell to produce mature secretory vesicles is probably found inside the endoplasmic reticulum (ER) of these cells, possibly also in the Golgi Apparatus (GA) and further extending up to the level of SV[4, 5].

14. ER is the main site for the post-translational processing of the most important molecules of the pancreatic beta cell: proinsulin and proamylin. There is proof (for example WFS1 gene whose expression generates an ER molecule, whose defect is responsible for the Rollison-Wolkot syndrome) that the beta cell ER (possibly also other cells could exhibit such „imperfections") cannot process correctly the two molecules with the consequent delay of selection of the molecules that will be sent to GA in order to generate nascent SV. Any defect of this chain of biochemical/ molecular processes can lead to the generation of SV incapable to completely maturate.

15. Diabetes mellitus is a disorder that starts many years before becoming apparent in the form of hyperglycemia. This is the reason I sustained for a long time that the diagnosis of diabetes mellitus should be based on other criterion than hyperglycemia alone. The decrease of the beta cell mass seem to be massive (~50%) at the moment of hyperglycemia onset, despite the fact that the number of studies supporting this affirmation (otherwise logical) are very few[16–19]. In theory, DM starts in the moment when an early apoptosed β cell cannot be replaced by a new one[20].

16. The relationship between the β cell function and the β cell mass is important but hard to prove since the access of researchers to the beta cell mass *in vivo* and *in situ*, is practically null. Moreover, this is limited also postmortem because of legislation that seems to protect the dead in the detriment of the living. However, the available data show a simultaneous (even if not parallel) deterioration of both β cell function and mass[21].

17. In the last decade, a number of researchers made justified efforts in order to understand the turnover of the β cells[18, 22] – if such a turnover does indeed exist. Recent studies[20] have challenged this view, demonstrating that the β cells are postmitotic cells, much like neurons, myocytes/ cardiomyocytes. From this moment on, any loss of β cell cannot be replaced. This means that in type 2 diabetic patients, the long duration of diabetes (sometimes more than 30 years) suggests that the process of losing β cells is a very slow one. This behavior of the human β cell is in striking contrast with the rodent or other animal models of diabetes. The excitable character of the β cell, that follows the law of stimulus → response, is sustained by its partially neuron-like phenotype.

18. We understand the interest for β cell regeneration as a promising replacement therapy[23]. However, it has been shown that, unlike in animal models that indeed show great pancreatic regenerative capacity throughout their entire lifespan, in humans this capacity has yet to be conclusively demonstrated, except, perhaps, in young age, when we encounter typical type I diabetes cases. A stimulation of regeneration in these cases is, theoretically, possible, but such cells appearing in an autoimmune milieu cannot mature in order to able to produce mature secretory vesicles. As we have shown before, in these patients proinsulin can be increased in their plasma, but C-peptide remains low, indicating that the insulin production is also very low.

19. The data reported above refer mainly to the T2DM phenotype which is probably not only the most frequent but also the most pathogenetically complex diabetes phenotype. It should be analyzed in the context of the contribution of an adipocyte defect to its pathogenesis, overweight T2DM subjects representing more than 85-90% of the cases[24, 25]. The importance of this factor is sustained by the parallelism between the epidemic of obesity and the pandemics of diabetes. Moreover, the un-precedent increase of T2DM in young only in the presence of obesity shows that weight excess would be the most important additive environmental factor that the global increase in T2DM prevalence.

20. The „explosion" of interest for the study of the adipocyte and adipose tissue, initiated in 1994 by the discovery of the endocrine function of this cell[26] will provide in the subsequent years and decades the necessary clarifications in order to explain an affirmation made by Paulescu 90 years ago: „*among others, most often the obese people become diabetics, as if the two diseases – obesity and fat diabetes – represent only two successive stages of the same pathological process*"[27]. We predict that this century will be the adipose tissue century.

21. From all the insulin-dependent cells, the adipocyte is the most exposed cell of the organism since the adipose tissue mass is the only one that can increase 5, 10, 30, 50× or even more. Slight increases (for example, the doubling of the adipose tissue mass from 3 to 6 kg) seem to have no visible pathologic consequence. Problems appear when the adipose mass increases more than 3-4 times. In this moment, a „*quiet*" adipocyte (with an important role in ensuring the energy homeostasis and immune balance of the human body) becomes, at first, „*restless"* (sending alert signals for all the tissues/organs involved in energy homeostasis) and finally an „*aggressive*" adipocyte when this peptide signals (hormonal, cytokinical or biochemical) have negative effects on the important cells involved in the regulation of energy metabolism.

22. The major importance of the adipose tissue, besides the pancreatic beta cell mass/function, in the pathogenesis of T2DM derives from the intrinsic physiologic characteristics of these two tissues: while the *β cell mass* has a natural tendency of decrease after reaching adult age[20, 28], the *adipose tissue* starts to increase progressively in those subjects that will become obese. When the progressively decreasing β cell mass is confronted with a progressively increasing adipose mass, diabetes will inevitably appear when the beta cell mass will reach the point when it cannot cope with providing completely the insulin required by the energy system, especially the tremendous number of adipocytes. For a short period of time insulin secretion can increase by the increased workload of an already decreased number of β cells. In these conditions, it is expected that the molecular transit through the ER of these cells will be accelerated, with the consequence of accentuating the pre-

existing defect in the processing of these two secretory molecules: proinsulin and proamylin. Even so, diabetes associated with obesity has very interesting evolutive particularities. One of these is represented by the reversibility of the β cell secretory defect. This was proved by the results of „bariatric surgery" that can lead to long term „remission" or „resolution" of the diabetes syndrome[29], a real phenomenon despite some exaggerations regarding its importance as „*a cure for diabetes*".

23. The diabetogenic role of obesity seems to be much more complex than we currently know. This role is sustained also by the genetic studies. Among the ~20 genes firmly associated with diabetes (identified by the recent GWA studies), a few are associated with the body mass (FTO, PPARG, MC4R, HHEX-IDE – the last one being associated with birthweight), sometimes with protective effects, while other times with pathogenic effects[30–34]. Some of the important genes (*TCF7L2* for example) are involved in the development of several tissues (pancreatic islets and adipose tissue among others), influencing both their development but also their future functional capacity[33, 34].

24. The role of the liver (hepatocyte) or skeletal muscle (miocyte) is secondary to the defects of the other two cells (β cells and adipocytes). These two tissues/organs (muscle and liver) have a small decisional role, being excellent execution organs: processing of fuels (inside the liver) and burning of fuels (inside the skeletal muscle).

25. The pathogenesis of T1DM is much clearer than that of T2DM, but still incompletely elucidated. It is based on the presence of a secretory β cell defect (we think it could be the same or similar with the proinsulin defect recorded in T2DM) and of a second defect in the immune function (especially the function of T lymphocytes with disequilibrium between the *Tregg* – aggressive and *Teff* cells – protective). The genetic basis of this phenotype is much better understood but it is neither necessary nor sufficient for the initiation of irreversible β cell autoimmunity[35]. The trigger of the autoimmune process is still incompletely elucidated, despite the fact that it was intensively studied especially among the environmental factors. We believe that the trigger could be found inside the pancreatic beta cell itself, *i.e.* the presence of the proinsulin processing defect. Since proinsulin is a strong beta cell antigen, its increase beyond a certain threshold could be the trigger of the autoimmune process[7].

26. The other diabetes phenotypes (MODY, neonatal diabetes) are „minor" as number of cases but important through the information they can provide as „monogenic models of the disease". Some of the genes involved in these forms of diabetes can be also found among the genes reported to be associated with T2DM[36].

27. An unexpected source of information is represented by the study of *lipodistrophy* syndromes, partial or total, inherited or acquired[37] that can shed new light on the real significance of insulin resistance. In order to be operational, this mechanism must have a clear molecular base. In the last years, insulin resistance was compromised as a primary pathogenic mechanism of T2DM, being at best a minor and secondary pathogenic factor. Unfortunately, many biochemical disturbances whose role in the pathogenesis of diabetes (such as oxidative stress, low-grade inflammatory response, obesity, and others) was automatically associated with the hypothetical insulin resistance, inducing a supplementary uncertainty in its real signification.

28. We are not denying the presence of a primary insulin resistance, especially that of „real insulin resistance" whose pathogenic mechanism is known: insulin receptor mutations/defects, anti-insulin antibodies and lipodystrophic disorders[38] and sometimes secondary to some drug administration[37]. However, we think it is not the case of type 2 diabetes for which the pathogenic events belonging in fact to obesity were falsely transferred to insulin resistance. We are not discussing the mechanisms through which obesity can induce secondary peripheral alterations interpreted as primary insulin resistance (pro-inflammatory reaction, decreased adiponectin, increased leptin, and increase in other adipokines with hormonal or citokininc function). In fact, in common T2DM, insulin resistance could be compared with the „shadow of obesity". When obesity disappears, evidently its shadow (insulin resistance) will disappear. Otherwise, how could a well-defined insulin resistance, confirmed by all the „golden standards" can disappear a few days or weeks after weight loss following bariatric surgery? And what is the answer of the geneticists to the cry from a recent paper: „Where *are the insulin resistance genes*?"[39], when the recent GWA studies could not identify at least one gene really associated with insulin resistance. When I first read

this rhetoric question, I could already provide myself the answer: „It would be indeed an extraordinary performance of genetics to identify the genes for an abstract concept".

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