Perspectives in Medicine
doi: 10.4183/aeb.2014.317

A CHALLENGE FOR THE AUTOIMMUNE DIABETOGENIC MECHANISM IN TYPE 1 DIABETES?

C. Ionescu-Tîrgovişte[*], P.A. Gagniuc[1,2,*], C. Guja[1,2]

"N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

Abstract

The pathogenesis of type 1 diabetes became a history longer and longer. There are 40 years since the immunogenetic theory of type 1 diabetes has been launched. Near this anniversary a challenge of this theory was recently published. We give here our interpretation of primary cause of type 1 diabetes which must be connected with the pathogenesis of other phenotypes of diabetes which has a main similar mechanism: the β-cell dysfunction.

Key words: diabetes, β-cell, T1D, autoimmune.

The year 2014 seems to become more important than a year of the 40th anniversary of the positing of the immunegenetic theory of type 1 diabetes (T1D) (1-3). It happened that end of 2013 and the beginning of 2014 was marked by the publication of several controversial papers, which require commentary. Moreso as these papers were directly related to the mechanism of β-cell destruction associated with classical autoimmune T1D recorded not only in pediatric patients, but with a lower frequency also in older age (4).

The most unexpected assertion comes from a reputed Swiss team Donath, Hess and Palmer (5) doubting the autoimmunity as the main mechanism operating in the destruction of the pancreatic β-cells in T1D. It may be a good opportunity for us to highlight several major deficiencies of the diabetological research in the last decades. Among these, the most prominent might be the plethora of experiments carried out on the NOD model of type 1 diabetes in mice, in sharp contrast with the small number of studies related to the pathogenesis of T1D using histological analysis of human pancreas. Donath *et al.* (5) were impressed by scarcity of histopathological documentation of "insulitis" collected and analyzed during a workshop by well-known experts of the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD). We learn from the Consensus release of this group that "Only 150-200 cases of insulitis have been described over the past century and very few of these cases have been analyzed in depth and with current methodologies" (6).

*Correspondence to: Constantin Ionescu-Tîrgovişte MD, PhD, "N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, I. Movila 5-7, Bucharest, 020475, Romania, E-mail: cit@paulescu.ro

Moreover, we learned that only now we have a consensual definition of "insulitis" as a lesion (a lymphocytic infiltration) with a minimum 15 CD45+ cells/islet in at least three islets. In a recent meta-analysis including the data obtained in children (0-14 years, with a duration of diabetes < 1 month) In't Veld (7) found that "insulitis' was present in only 73% of children. In older diabetics (15-39 years), the frequency of insulitis was even lower, of only 29%. These data seems to be in contradiction with the diagnosis of autoimmune T1D attested in children, adolescents and young adults if the islets antibodies were present and fasting or stimulated C-peptide levels, low (8). So, where is the truth? Is Donath *et al*. (5) right in questioning the autoimmune mechanism explaining the β-cell destruction?

Before answering this question, we added another recent publication from a reputed Danish group (Storling 2013 9), contesting the classical view of T1D as an autoimmune disease. The main argument for raising such a question was that the only specific antigenic molecule from β-cells is insulin. Indeed, the first antibodies which appear in the early phase of diabetogenesis, are anti-insulin/proinsulin antibodies (9-12). Why is there a spreading of the autoimmune reaction to other antigenic molecules from the β-cell (such as glutamic acid dehydrogenase (GAD), insulinoma associated protein 2 (IA2) or the zinc transporter 8 (Zn-T8)), could not have the same pathogenetic significance? Instead, these authors suggest as an immune (not autoimmune) anti-β-cell mechanism, a hypothetical post-translational modification of some β-cell molecules which became "neo-epitopes" with antigenic properties. The anti β-cell immune reaction against such non-self molecules might be the possible "triggers" of the destructive immune reaction. Such a mechanism has been reported as operational in various autoimmune diseases, like rheumatoid arthritis or multiple sclerosis, among others (13). In an accompanying commentary, Ake Lernmark (14) appreciates this hypothesis is suitable to rise a scientific debate, but it has no "strong experimental evidence".

To conclude, the "trigger" of the anti-β-cell autoimmunity remains a hot topic, having a great importance in deciphering the "first diabetogenic movement". We have advanced our view in this respect several years ago (15).

Returning to the Donath's contest

The main argument against the autoimmune mechanism of T1D is because "is one of the few remaining autoimmune diseases without any approved immunological treatment" (5). It is true that all tested anti-immune treatments shortly mentioned by them were unsuccessful. Is that observation enough to doubt the autoimmune mechanism of T1D? In our view, others are the causes for the failure of all attempts in stopping the autoimmune process. First, it refers to the belief that the treatments efficient in NOD mice might be also efficient in human autoimmune diabetes. However, the mice model of T1D is different in many respects. For instance, in mice the islets display massive leukocyte infiltration, whereas in humans, the islet infiltration

is limited to only a fraction of islets. The second reason is even more important: all attempts to prevent or to treat T1D in humans were applied in newly discovered diabetic patients, sometimes up to 5 years after onset! In such cases the β-cell mass is already irreversibly lost up to 80% from the initial value (16, 17). In addition, in contrast with the obvious regenerative capacity of β-cells in mice, this is practically lost in humans (18, 19). To conclude, all "preventive" or "therapeutic" attempts were applied too late.

Limited access to human pancreas

The small quantity of good quality pancreatic tissue obtained post-mortem has stimulated the imagination of generations of diabetologists in order to find alternative sources of *in vivo* bioptic material. Imagawa (20) proposed *in vivo* pancreatic biopsies using ultra-fine needles and echography guidance. Due to either blank procedure or a too small pancreatic tissue obtained, this method has been abandoned.

More recently, the despair of researchers facing the impossibility to investigate the pathophysiological phenomenology hidden inside the pancreatic islets led to the daring but laudable initiative of a North-European research Group (Norwegian, Danish, Swedish and Finn researchers), to perform a large enough pancreatic biopsy in living adults with recent-onset T1D (21). The procedure was performed in 6 cases using a modern laparoscopic technique. We would be happy to be a part of this team, as maybe Mark Atkinson also would have wished considering his positive commentary regarding this "in extremis" tentative (22). As Atkinson, we wait with great interest the results of a more in depth histological analysis, which can inform us on several controversial features regarding the extension of the insulitis process, at least at the moment when good quality pancreatic tissue will become available. We hope to obtain more data regarding the conflict between the β-cells and the cells of the immune system.

We mention that the whole bioptic procedure of the Northern-European group was carried out in the framework of DiViD (Diabetes Virus Detection) Study, which was successful in investigating the controversial topic of virus infection as a potential trigger of anti-β-cell autoimmunity (23). For ethical reasons, this study included only adult patients aged between 24 and 35 years. The duration of diabetes at the moment of this procedure was of one month. The Study has been approved by ethical committees of the participant institutions and for each participant an informed consent has been obtained (21).

Pathogenesis of T1D in the light of the histological features

In our view, the immune/autoimmune mechanism specifically targeting the pancreatic β-cells is supported by too many arguments to be challenged. If this mechanism is a primary one (the direct consequence of a disturbed immune system) or a secondary one (triggered by a concomitant β-cell defect) it is still a matter of debate. Our point of view, sustained for many years (24-32), is that T1D and all its sub-phenotypes (32,31) results from two concomitant, conver-

gent and genetically determined disturbances. Both are necessary for inducing the T1D phenotype of diabetes, but their individual contribution in the destruction of the pancreatic β-cells might be different from a patient to another according to the inherited genetic architecture and the environmental (epigenetic) influences.

Our initial hypothesis started from the observation that in offspring/siblings of T1D patients, the level of plasma proinsulin or the proinsulin-to-insulin ratio are increased (33-37). Such an abnormal increase clearly expresses the inability of the pancreatic β-cells to produce mature Secretory Vesicles (SV) (Fig. 1), the only ones that can respond promptly and efficiently to their physiological stimuli (29,30). In fact, the normal β-cell is not an "insulin factory" (38) but a "mature SV factory" (30). In contrast, the immature SV are associated with several other secretory defects (disappearance of oscillatory insulin secretion and the decrease of the first phase of insulin secretion (39). These alterations can be detected several years before the onset of T1D. Very important, the secretory defects are contemporary with the increased levels of anti-β-cell antibodies (40). Moreover, Skowera *et al.* (41) have identified in the signal peptide of the pre-proinsulin molecule a glucose-regulated epitope with antigenic properties that can be detected by circulating effector CD8+ T cells. They demonstrated also that cloned pre-proinsulin signal peptide-specific CD8+ T cells were able to kill human β-cells *in vitro* (41).

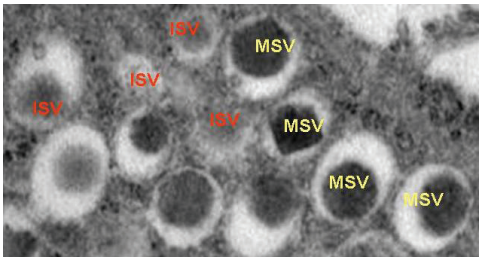

Figure 1. Heterogeneity in the maturation of the secretory vesicles; MSV (mature secretory vesicles) and ISV (immature secretory vesicles).

For us, it is obvious that these defects can be sensed by a hypersensitive/labile immune system, increasing the ratio between T cytotoxic cells (Teff) and regulatory T cells (Treg) (8). There are strong arguments favoring the hypothesis that the trigger of the autoimmune attack against β-cells could be found in the structural defect in the membrane of the immature SVs and correlated with the higher proinsulin levels inside them (42, 29).

It is interesting to note that in the molecular structure of the SVs membrane have been identified some specific antigenic molecules (Roep *et al.* 2003, 41, 43), apart the classical β-cell antigens: proinsulin/insulin, GAD, IA2 and Zn-T8 (11, 45). The last antigen (Zn-T8) is closely related to the SVs membrane (46,47). Although the gene encoding the zinc transporter molecule (SLC30A8) has been found as associated with T2D (48), the antibodies against Zn-T8 are detected in association with T1D (11,45).

Autoimmune destruction of the pancreatic β-cells

All the above mentioned changes in the structure and function of the SVs cannot be ignored by the "immune sensors", probably found in many if not in all immune cells. We do not have direct evidence on what happens inside the

islet cells and in the immune cells in the early phases of human T1D. However, using an ingenious method (the transplantation of a normal islet in the anterior chamber of the eye of a NOD mice), several authors (49, 50) had the opportunity to perform a non-invasive imaging of the complex dynamic process of autoimmune diabetes. Using this technique, they observed the essential role of a subset of Dendritic Cells (DC). Also recently and using a tridimensional imaging of mice islets, Tang *et al.* (51) demonstrated the role of the glio-endothelial barrier (formed by Schwann- glial cells and the endothelial associated pericytes), reacting to any islet lesion, including that associated with peri-insulitis.

A subset of macrophages able to produce proteases of cathepsine type may play a key role in the destruction of peri-capillary basal membrane (52). By the brakes made in the peri-insular glio-endothelial capsule, the first immune cells enter inside the islet, mediating the transformation of peri-insulitis in a process of intra-insulitis. According to the video-cinematographic recordings, Schmidt-Christiansen *et al.* (50) observed that within a time span of 7 days, a subset of CD11c+ cells (with typical DC morphology) were recruited inside the islets. They remain stationary, playing an active role by protruding and retracting their dendrites into the islet parenchyma. Their main role seems to be that of probing and sampling molecules (antigens) or structures (secretory vesicles), and transferring the information to T cytotoxic cells. The specificity of the β-cell destruction inside the islet cells suggests that immune sensors will detect only the defect cells, those containing immature SVs and rich in proinsulin. Because DC sends also this information to the B lymphocytes, these will produce specific antibodies to all antigenic molecules detected by the DCs.

The above mentioned arguments suggest that the β-cell destruction has as a main particularity the fact that the main driver of the destructive process operating inside the "multi-cells" islets only the pancreatic β-cells contaning secretory defect, act as a "physiologic" trigger of the autoimmune reaction.

A powerful argument in favor of the autoimmune mechanism acting in T1D is that of the genetic base of this phenotype. A high number of the genes found to be associated with T1D (53-55) are closely related with the immune system. The first 6 genes associated with T1D (explaining ~70% of the heritability of T1D) include the HLA complex (2, 54), CTLA4 (56,57), PTPN22 (58), IL-2RA (58) and IFIHI (54). The last, INS (59) has not a direct relation with immunity. Because its VNTR region contains a locus also associated with T2D (60), we think that this gene could be related either with the β-cell function but also with their antigenic function. The same significance might have the SLC30A8 gene encoding the zinc transporter (Zn-T8). Its association with T1D is obvious as the antibodies against Zn-T8 are included now among the immune markers for this phenotype.

The last argument in favor of the immunogenetic theory of T1D is the recently published paper of Ziegler *et al.* (45). This will remain a landmark long-term prospective study detecting careful-

ly the early markers of T1D and their real value in predicting the evolution towards clinical diabetes. This well conceived and well conducted multi-centric study enrolled a total of 13,377 newborns with high risk for diabetes, recruited from Germany, Finland and Colorado (USA). All subjects were regularly tested (with small differences between centers) from birth up to 25 years. The German cohort of this study enrolled patients starting with 1988 up to 2012. The main steps in diabetes progression that were analyzed were: the first sero-conversion, the conversion to multiple antibodies and the clinical onset of diabetes. A strong correlation has been found between the seroconversion to at least two antibodies or three antibodies (Fig. 2) and progression towards clinical onset. The Zn-T8 antibodies were not available at the beginning of the study and were not included in the analysis.

The main characteristic resulting from this study is the large heterogeneity regarding the moment of first serological conversion, the second or third seroconversion and the clinical onset of diabetes. In a few cases, the evolution to seroconversion and then to clinical diabetes was rapid (several months or a few years), whereas in others this process took more than 15 or 20 years. This heterogeneity corresponds with other data (Fig.3), starting with anatomo-histological features of the pancreas, the number, dimension and distribution of islets inside the pancreas, the number of β-cells in an islet, their various function and various vulnerability, and many other features (28-30, 32, 61-64). In other words, there are many mysteries hidden in this "black box", which was the main impulse for the courageous approach of Krogvold *et al.* (21).

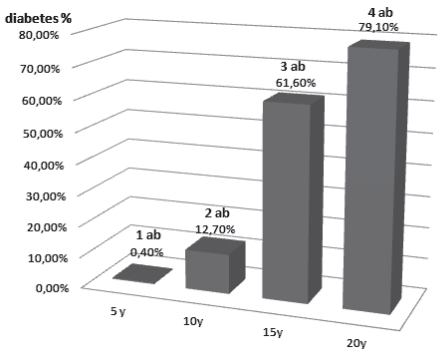

Figure 2. The prevalence of diabetes increases with the number of antibodies from 0.40% in subjects with only one antibody up to 79.10% in subjects with positivity for 4 antibodies, in 403 progressors to diabetes, in terms of the duration of observation. Figure adapted after data published by Ziegler *et al.* 2013 (44).

We have to mention that, despite the great efforts for developing an imagistic method able to assess the β-cell mass (65) or insulitis process (66), this aim has not been achieved yet. It is obvious that the images offered by such methods could not be complete without the images obtained on fresh tissue, even if this analysis is made *ex vivo* and on a small number of cases. Probably new image processing algorithms can provide new insights into the future (67).

The risk of the in vivo bioptic approach of the pancreas

We come back to the initiative of the North-European group and to the unpredicted technical problems encountered. From the 6 biopsied cases, in 3 cases some surgical complications appeared. One of them (an important bleeding caused by a spleen lesion), we

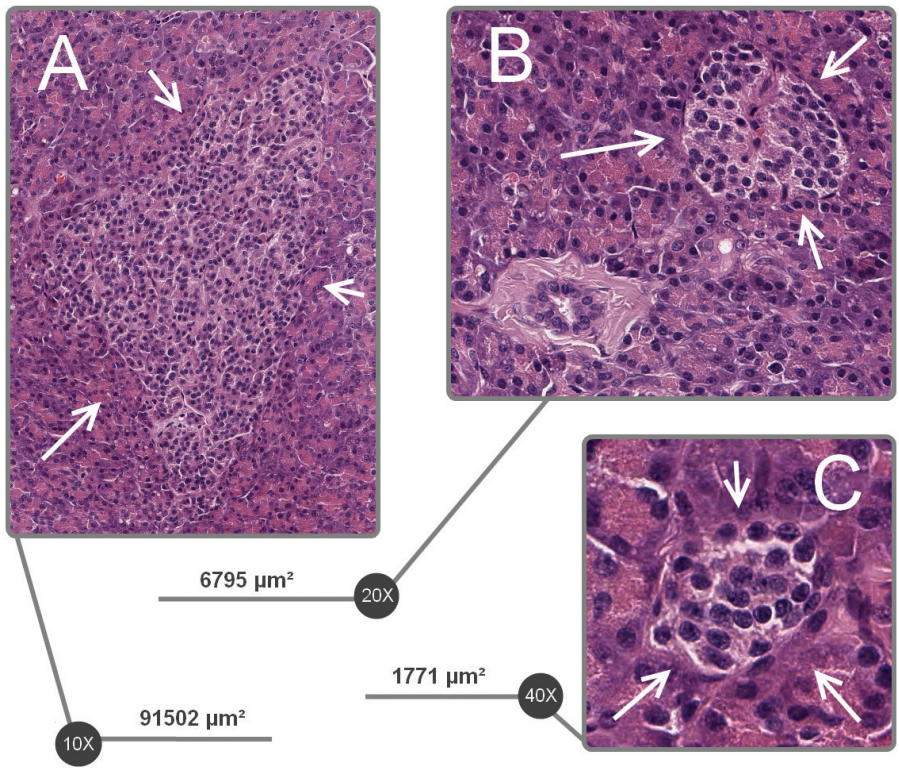

Figure 3. The heterogeneity of islets cells according to their surface; (A) big islets, (B) medium islets and (C) small islets.

believe that it could have been avoided. It remains the two cases in which a post-surgical leakage of pancreatic juice from the margin of resection occurred. These complications were solved by a several days of careful medical treatment.

The question is "could these incidents have been avoided?". In his comment, Atkinson (22) raised an interesting question to which we have to find an answer. "Why did complications arise in some patients and not in others?" The answer we try to formulate refers to the attentive analysis of the quality of "pancreatic capsule", more precisely of its strength. In contrast with other parenchymatous organs, (kidney or liver for example), the pancreas does not possess a similar capsule, but rather a more loose fibro-conjunctive cover which, in very thin subjects, allows identifying the pancreatic lobules through its transparency. Sometimes adipose cells can be included in this fibro-conjunctive sheet.

The answer to the Atkinson question could be related to the nature of this pancreatic capsule. The leakage of pancreatic juice from the surgical suture of the remnant pancreas could be the consequence of the lack of strength of this capsule. It seems that the pancreatic capsule is more resistant in the region of the pancreatic head than for the tail of this organ. If the fragility of the pan-

creatic "pseudo-capsule" will be proven to be the cause of the surgical complications mentioned in the DiViD study, two potential solutions can be taken into account in order to avoid these complications: either an attentive visual evaluation of the consistency of the pancreatic capsule, abandoning the procedure if the surgical suture seems difficult, or a larger pancreatectomy to include both the tail and the body of the gland. Obviously this alternative could be rejected by the majority of patients. However, we think that this will not influence the insulin requirements in already established T1D, while the remnant tissue could provide enough exocrine pancreatic juice in order to prevent malabsorption. Another alternative could be to find a bio-compatible material to cover the surface of the remnant pancreas, avoiding the risk of leakage.

Ethical issues in scientific research

The key to the current discussion excludes the pancreatic biopsy approach from research only. We refer to not out of mindless curiosity as in the myth of Pandora's Box (22). We are convinced that the Northern European researchers were well aware of the risks they took when performing a large pancreatic biopsy. At the same time, we all know that the information obtained by their approach could lead to significant pathogenic informations applicable to the future generations of T1D patients. We consider that performing such a daring intervention demands a superior level of research, which depends on finding an efficacious preventive method in T1D, impossible without better knowing the first steps of the diabetogenic process. Up to now, research has been limited to indirect methods already mentioned above. These indirect methods proved to be inaccurate and with mixed informations regarding β-cell secretory dysfunction and the immune reaction. Unfortunately, most often the study of the pancreatic β-cell function and that of the autoimmune reaction was done separately, making difficult to understand their pathogenic interconditioning. There are only a few papers in which (besides the assessment of the anti β-cell antibodies) β-cell function was also evaluated, considering that this is only a direct consequence of the autoimmune process. In fact, logic tells us that the causal pathogenic relationship could be reversed. Thus, the primary defect could be the β-cell dysfunction, maybe the only one capable of getting the immune system involved, which, due to as yet fully unfathomed reasons, starts to attack β-cell structure even if these are not deteriorated to the level that would determine their removal from the body.

What is now required is to precisely establish the chronology of the pathogenic steps in T1D evolution, maybe by careful parallel analysis of descendants of diabetic parents following the excellent model developed by Ziegler *et al.* (44) for the study of autoimmunity. Still, it will remain an issue of the dynamics of morphological changes that take place inside the pancreatic islets. For the moment, we do not know why, where and in how many islets the insulitis process occurs. We also do not know the speed of its evolution. Conse-

quently, we do not know yet when and how to intervene to prevent the conflict between the pancreatic β-cell and the immune system. The decades long failure of the preventive interventions after the clinical onset of the disease represent only another proof that these attempts had, in the best case scenario, only the characteristics of secondary or even tertiary prevention (5).

This is one of the reasons for which we support the North-European group (21) that opened Pandora's box, but did not close it back (as Pandora did). This is why hope will be our companion in conquering a castle that, maybe, will prove not to be the last.

We dream of preventing autoimmune T1D much more than we dream of preventing the much more common Type 2 diabetes. The latter depends more on the chaotic development of human society and its economic model during the last century, a pattern that realistically cannot be changed too soon. On the other hand, with the immune system we could negotiate a "non-aggression pact". For these we only need skilled "diplomats".

Conflict of interest

We declare that there is no conflict of interest.

Acknowledgement

We thank the Fundeni Institute Clinic (Prof. Irinel Popescu and Simona Olimpia Dima, MD, PhD) for providing pancreatic tissue and also Nicolae Mirancea, PhD for processing samples of pancreatic tissue and analysis by electron microscopy (Fig.1). 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

1. Nerup J, Andersen OO, Bendixen G, Egeberg J, Poulsen JE. Anti-pancreatic cellular hypersensitivity in diabetes mellitus. Diabetes 1971; 20:424-427.

2. Nerup J., Platz P, Andersen OO, Christy M, Lyngsoe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A. HLA antigens and diabetes mellitus. Lancet 1974; ii:864-866.

3. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune plyendocrine deficiencies. Lancet 1974; 2:1279-1283.

4. Ionescu-Tîrgovişte C, Paterache E, Cheţa D, Farcaşiu E, Serafinceanu C, Mincu I. Epidemiology of diabetes in Bucharest. Diabetic Med 1994; 11:413-417

5. Donath MY, C Hess, E Palmer. What is the role of autoimmunity in type 1 diabetes? A clinical perspective. Diabetologia 2014; 57: 653-655.

6. Campbell-Thompson ML, Atkinson MA, Butler AE, Chapman NM, Frisk G, Gianani R, Giepmans BN, von Herrath MG, Hyoty H, Kay TW, Korsgren O, Morgan NG, Powers AC, Pugliese A, Richardson SJ, Rowe PA, Tracy S, In't Veld PA. The diagnosis of insulitis in human type 1 diabetes. Diabetologia 2013; 56(11):2541-2543.

7. In't VP, Lievens D, De GJ, Ling Z, Van der Auwera B, Pipeleers-Marichal M, Gorus F, Pipeleers D. Screening for insulitis in adult autoantibody-positive organ donors. Diabetes 2007; 56(9):2400-2404.

8. Eisenbarth GS. Type 1 diabetes mellitus. A chronic autoimmune disease. New Engl J Med 1986; 314:1360-1368.

9. Storling J , Aj Overgaard, CA Brorsson, F Piva, CH Bang-Berthelsen, C Haase, J.Nerup, F Pociot: Do post-translational beta cell protein modifications trigger type 1 diabetes. Diabetologia 56:2347-2354, 2013

10. Achenbach P, Koczwara K, Knopff A, Naserke H, Ziegler AG, Bonifacio E. Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. J Clin Invest 2004; 114(4):589-597.

11. Bingley P.J, Gale EA. Progression to type 1 diabetes in islet cell antibody-positive relatives in the European Nicotinamide Diabetes Intervention Trial: role of additional immune, genetic and metabolic markers of risk. Diabetologia 2006; 49:881-890.

12. Ziegler AG, Hummel M., Schenker M, Bonifacio E. "Appearance and risk for

development of childhood diabetes in offspring of parents with type 1 diabetes". Diabetes 1999; 48:460-468.
13. Dunne J.L., L. Overbergh, A.W. Purcell, C Mathieu. Posttranslational Modifications of protenis in type1 diabetes: The next step in finding the cure?: Diabetes 2012; 61: 1907-1914.
14. Lernmark A. Is there evidence for post-translational modifications of β cells autoantigens in the aetiology and pathogenesis of type 1 diabetes. Diabetologia 2013; 56: 2355-2358.
15. Ionescu-Tîrgovişte C. Proinsulin as the possible key in the pathogenesis of type 1 diabetes. Acta Endocrinol (Buc), 2009; 5, 233-249.
16. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52(1):102-110.
17. Meier JJ, RC Bonadonna. Role of reduced β-cell mass versus impaired β-cell function in the pathogenesis of type 2 diabetes. Diabetes Care 2013; 36 (Suppl2): S113-S120.
18. Maedler K., Schumann D., Schulthess F., Oberholzer J., Bosco D., Berney T., Donath M.Y.: Aging correlates with decreased β-cell proliferative capacity and enhanced sensitivity to apoptosis. A potential role for Fas and pancreatic duodenal home box- 1. Diabetes 2006; 55, 2455-2462.
19. Cnop M., Hughes S.J., Igoillo-Esteve M., Hoppa M.B., Sayyed F., Van de Laar L., Gunter J.H., Koning E.J.P., Walls G.V., Gray D.W.G., Johnson P.R.V., Hansen B.C., Morris J.F., Pipeleers-Marichal M., Cnop I., Clark A. The long lifespan and low turnover of human islet β cells estimated by mathematical modeling of lipofuscin accumulation. Diabetologia 2010; 53, 321-330.
20. Imagawa A, Hanafusa T, Tamura S, Moriwaki M, Itoh N, Yamamoto K, Iwahashi H, Yamagata K, Waguri M, Nanmo T, Uno S, Nakajima H, Namba M, Kawata S, Miyagawa JI, Matsuzawa Y. Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. Diabetes 2001; 50(6):1269-1273.
21. Krogvold L, Edwin B, Buanes T, Ludvigsson J, Korsgren O, Hyoty H, Frisk G, Hanssen KF, Dahl-Jorgensen K. Pancreatic biopsy by minimal tail resection in live adult patients at the onset of type 1 diabetes: experiences from the DiViD study. Diabetologia 2014; 57(4):841-843.
22. Atkinson MA. Pancreatic biopsies in type1 diabetes: revisiting the myth of Pandora`s box. Diabetologia 2014; 57:656-659.
23. Yeung WC, Al-Shabeeb A, Pang CN, Wilkins MR, Catteau J, Howard NJ, Rawlinson WD, Craig ME. Children with islet autoimmunity and enterovirus infection demonstrate a distinct cytokine profile. Diabetes 2012; 61(6):1500-1508.
24. Ionescu-Tîrgovişte C, Guja C, Ioacara S, Vladica M.: Plasma proinsulin could be a marker of β cell dysfunction in both type 2 and type 1 diabetes, Diabetic Med. 2006; 23, (Suppl.4): 66-67.
25. Ionescu-Tîrgovişte C., Guja C., Mota M., Ioacara S., Vladica M., Pascu M., Mihai A.: Plasma proinsulin levels in long standing diabetes: marker for a dysfunctional β cell regeneration? [Abstract]. Diabetes 2007; 56 (Suppl.1) A423.
26.Ionescu-Tîrgovişte C. For a new paradigm of diabetes. Rom J Intern Med 2007; 45:3-15.
27.Ionescu-Tîrgovişte C., Guja C. Proinsulin, proamylin and the β cell endoplasmic reticulum: The key for the pathogenesis of different diabetes phenotypes. Proc. Rom. Acad., Series B, 2007; 2, p. 113–139.
28.Ionescu-Tîrgovişte C, Guja C. The various phenotypes of diabetes and the endoplasmic reticulum of the β cell. Rom J Intern Med. 2007; 45(3):287-291.
29. Ionescu-Tîrgovişte C. Proinsulin as the possible key in the pathogenesis of type 1 diabetes. Acta Endocrinol (Buc), 2009; 5, 2: 233-249.
30. Ionescu-Tîrgovişte C. Insulin Resistance - What is Myth and What is Reality? Acta Endocrinol (Buc) 2011; 7, 2: 123-146.
31. Ionescu-Tîrgovişte C., Gagniuc P, Guja C. The promoters of genes associated with type 1 and type 2 diabetes seem to have some specific features. Diabetologia,2012; 55, Supplement 1, pp 1-538.
32. C. Ionescu-Tîrgovişte. To limit the black and white view on diabetes. Acta Endocrinol (Buc), 2013; 9, 4: 597-604.
33. Srikanta S, Ganda OP, Rabizadeh A, Soeldner JS, Eisenbarth GS. First-degree relatives of patients with type I diabetes mellitus. Islet-cell antibodies and abnormal insulin secretion. N Engl J Med 1985; 313(8):461-464.
34. Hartling SG, Lindgren F., Dahlqvist G., Persson B, Binder C.: Elevated proinsulin in healthy siblings of IDDM patients independent of HLA identity. Diabetes 1989; 38:1271-1274.
35. Lo SS, Hawa M, Beer SF, Pyke DA, Leslie RD. Altered islet β-cell function before the onset of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1992; 35(3):277-282.

36. Røder ME, Knip M., Hartling SG, Karjalainen J., Åkerblom HK, Binder C., The Childhood Diabetes in Finland Study Group. Disproportionately elevated proinsulin levels precede the onset of insulin-dependent diabetes mellitus in siblings with the low first phase insulin responses. J. Clin Endo Metab 1994; 79:1570-157.
37. Røder ME, Porte D, Schwartz RS, Kahn SE. Disproportionately elevated proinsulin levels reflect the degree of impaired β-cell secretory capacity in patients with non-insulin dependent diabetes mellitus. J Clin Endo Metab 1998; 83: 604-608.
38. Orci L.: Macro- and micro-domains in the endocrine pancreas. Diabetes 1982; 31:528-546.
39. Rorsman P, M Braun. Regulation of insulin secretion in human pancreatic islets. Ann rev Physiol 2013; 75:175-179.
40. Truyen I, De PP, Jorgensen PN, Van SC, Ubani O, Decochez K, Vandemeulebroucke E, Weets I, Mao R, Pipeleers DG, Gorus FK. Proinsulin levels and the proinsulin:c-peptide ratio complement autoantibody measurement for predicting type 1 diabetes. Diabetologia 2005; 48(11):2322-2329.
41. Skowera A, Ellis RJ, Varela-Calvino R, Arif S, Huang GC, Van-Krinks C, Zaremba A, Rackham C, Allen JS, Tree TI, Zhao M, Dayan CM, Sewell AK, Unger WW, Drijfhout JW, Ossendorp F, Roep BO, Peakman M. CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. J Clin Invest 2008; 118(10):3390-3402.
42. Despa F., Ionescu-Tîrgovişte C. Accumulation of toxic residues in β-cells can impair conversion of proinsulin to insulin via molecular crowding effects. Proc. Rom. Acad. Series B 2007; 3:225-233.
43. Roep B.O.: Islet autoreactive CD8 T-cells in type 1 diabetes. Licensed to kill? Diabetes 2008; 57: 1156-1157.
44. Ziegler AG, Pflueger M, Winkler C, Achenbach P, Akolkar B, Krischer JP, Bonifacio E. Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children. J Autoimmun 2011; 37(1):3-7.
45. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013; 309(23):2473-2479.
46. Chimienti F., Libert T., Pointecouteau P. The Zinc transporter ZnT8 protects cultured β cells from oxidative stress-induced cell death. Diabetologia 2007; 50 (Suppl 1) : A 142 (S 64).
47. Wenzlau JM, Liu Y, Yu L, Moua O, Fowler KT, Rangasamy S, Walters J, Eisenbarth GS, Davidson HW, Hutton JC. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 2008; 57(10):2693-2697.
48. Sladek R., Rocheleau G., Rung J., Dina C., Shen L., Serre D., Boutin Ph., Vincent D., Belisle A., Hadjadj S., Balka B.u, B. Heude, G. Charpentier, T. J. Hudson, A. Montpetit, A.V. Pshezhetsky, M. Prentki, B.I. Posner, Balding D. J., D. Meyre, C. Polychronakos, Ph. Froguel. A genome-wide association study identifies novel risk loci for type 2 diabetes Nature 2007; 445, 881-885.
49. Abdulreda MH, Faleo G, Molano RD, Lopez-Cabezas M, Molina J, Tan Y, Echeverria OA, Zahr-Akrawi E, Rodriguez-Diaz R, Edlund PK, Leibiger I, Bayer AL, Perez V, Ricordi C, Caicedo A, Pileggi A, Berggren PO. High-resolution, noninvasive longitudinal live imaging of immune responses. Proc Natl Acad Sci U S A 2011; 108(31):12863-12868.
50. Schmidt-Christensen A, Hansen L, Ilegems E, Fransen-Pettersson N, Dahl U, Gupta S, Larefalk A, Hannibal TD, Schulz A, Berggren PO, Holmberg D. Imaging dynamics of CD11c(+) cells and Foxp3(+) cells in progressive autoimmune insulitis in the NOD mouse model of type 1 diabetes. Diabetologia 2013; 56(12):2669-2678.
51. Tang SC, Yc Chiu, CT Hsu, SJ Peng, YY Fu. Plasticity of Schwann cells pericytes in response to islet injury in mice. Diabetologia 2013; 56: 2244-2434.
52. Korpos E, Kadri N, Kappelhoff R, Wegner J, Overall CM, Weber E, Holmberg D, Cardell S, Sorokin L. The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. Diabetes 2013; 62(2):531-542.
53. Erlich H. A., A M Valdes, J.A. Noble. Prediction of Type 1 diabetes. Diabetes 2013; 62:1020-1021.
54. Todd JA. Etiology of type 1 diabetes. Immunity 2010; 32:457-467.
55. Guja C, Gagniuc P, Ionescu-Tîrgovişte C. Genetic factors involve in pathogenesis of type 2 diabetes, Proc. Rom. Acad., Series B 2012, 1, p. 44–61.
56. Guja C., Todd JA, Smith A, Welsh K, Merriman

M, Smith A, Todd JA, Ionescu-Tîrgovişte C.: The study of CTLA4 and Vitamin D receptor polymorphisms in Romanian type 1 diabetes population. J Cell Mol Med 2002; 6: 75-81.
57. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bing-ley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tîrgovişte C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003; 423:506–511.
58. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009; 360:1646-1654.
59. Barratt BJ , Payne F , Lowe CE, Hermann R., Healy BC, Harold D, Concannon P, Gharani N, McCarthy MI, Olavesen MG, McCormack R, Guja C, Ionescu-Tîrgovişte C, Dag E Undlien, Rønningen KS, Gillespie KM, Tuomilehto-Wolf E, Tuomilehto J, Bennett ST, Clayton D, Cordell H, Todd J. Remapping the insulin gene / IDDM2 locus in type 1 diabetes. Diabetes 2004; 53:1884-1889.
60. Huxtable SJ, Saker PJ, Haddad L, Walker M, Frayling TM, Levy JC, Hitman GA, O'Rahilly S, Hattersley AT, McCarthy MI. Analysis of parent offspring trios provides evidence for linkage and association between the insulin gene and type 2 diabetes mediated exclusively through paternally transmitted class III variable number tandem repeat alleles. Diabetes 2000; 49(1):126-130.
61. Pipeleers DG: Heterogeneity in pancreatic β cell population. Diabetes 1992; 41:777-781.
62. Pipeleers D., Ling Z.: Pancreatic β cells in insulin-dependent diabetes. Diabetes Metab Rev 1992; 8:209-227.
63. Pipeleers D, Kiekens R, Ling Z, Wilikens A, Schuit F. Physiologic relevance of heterogeneity in the pancreatic β cell population. Diabetologia (Suppl.2) 1994; 37:S57-S64.
64. Pipeleers D, Hoorens A, Marichal-Pipeleers M, de Casteele MV, Bouwens L, Ling Z. Role of pancreatic β-cells in the process of β-cell death. Diabetes 2001; 50, Suppl.1, S52.
65. Brom M, van der Weg WW, Joosten Lieke, Friedlink C, Bouckenooghe T, Rijken P, Andralojc K, Goke BJ, de Jong M, Eizirik DL, Behe M, Lahoutte T, Oyen WIG, Tack CJ, Janssen M, Boerman OC, Gotthardt M. Non-invasive quantification of the β-cell mass by SPECT with 111 In-labelled exendin. Diabetologia 2014; 57(5) 950-959.
66. Gialleonardo V, Vries EF, Di Girolamo M, Quintero AM, Dierckx RAJO, Signore A. Imaging of β-cell mass and insulitis in insulin-dependent (type 1) diabetes mellitus. Endo. Rev 2012; 33(6): 892-919.
67. Gagniuc and Ionescu-Tîrgovişte. Gene promoters show chromosome-specificity and reveal chromosome territories in humans. BMC Genomics 2013; 14:278.