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Insulin secretion in the early phase of type 1 diabetes mellitus (T1DM) and new hopes for maintaining it through therapy

Insulinosekrecja we wczesnej fazie cukrzycy typu 1 i nowe nadzieje terapeutyczne na jej utrzymanie

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Podziękowania
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Abstract
Type 1 diabetes mellitus (T1DM) is an autoimmune disease with a progressive loss of pancreatic beta cell functional mass and subsequent impairment of insulin secretion. At present, the most important factors that help sustain insulin secretion at the early stage of the disease and have the potential to reduce the risk or even prevent long-term diabetic complications include early diagnosis, early initiation of the insulin therapy, an appropriate education of patients and immunotherapeutic interventions. In this paper, the issue of insulin secretion at the early stage of T1DM and some of the most recent research on novel therapies supporting traditional treatments are discussed.
Key words:
type 1 diabetes, insulin secretion, clinical remission, beta cells, immune intervention

Streszczenie
Cukrzyca typu 1 (T1D) jest chorobą autoimmunologiczną, w której przebiegu dochodzi do utraty funkcjonalnej masy komórek beta trzustki i stopniowego zaniku insulinosekrecji. Wczesne rozpoznanie T1D, szybkie wdrożenie leczenia insuliną, odpowiednia edukacja chorych oraz immunointerwencje terapeutyczne – to obecnie najbardziej istotne czynniki, które poprzez wydłużenie okresu insulinosekrecji we wczesnym etapie choroby mogą w przyszłości zmaksymalizować szansę na redukcję ryzyka, a nawet uniknięcie powikłań przewlekłych cukrzycy. Artykuł stanowi omówienie zagadnienia insulinosekrecji w początkowych fazach T1D i wybranych najnowszych badań nad opracowaniem terapii wspomagających tradycyjne leczenie.
Słowa kluczowe:
cukrzyca typu 1, insulinosekrecja, remisja kliniczna, komórki beta, immunointerwencja

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Introduction

Type 1 diabetes mellitus (T1DM) results from an autoimmune destruction of pancreatic beta – cells leading to an absolute insulin deficiency. The process is induced by environmental factors in persons with particular genetic predispositions, mainly linked with the HLA system. Before the disease manifests itself openly, there is a long-lasting (for months or even years) preclinical (latency) phase with a progressive loss of functional beta-cells mass resulting from their destruction by an autoimmune process. The process encompasses both humoral and cellular mechanisms. Sensitized T lymphocytes destroy beta cells by recognising their specific antigens. This autoimmune process (insulitis) is caused mainly by lymphocytes T infiltrating pancreatic islets, but it is also accompanied by serum auto-antibodies appearing early and posing an excellent diagnostic tool, enabling for the diagnosis already in the latency (preclinical) phase. Auto-antibodies against pancreatic antigens include GAD65 (Anti-Glutamic Acid Decarboxylase), ICA512 (anti-islet cells), IAA (Insulin AutoAntibodies), ZnT8 (Zinc Transporter 8 Antibodies) and IA2 (Islet Antigen 2 Antibodies) [1]. The latency phase is symptomless so the T1DM diagnosis in this period is usually accidental. Most often the disease is being diagnosed when typical signs and (polydipsia, polyuria or weight loss) develop, i.e. at the time when about 85-90% of the insulin-producing cells have already been destroyed [2, 3]. The remaining beta-cells allow some patients to preserve residual insulin secretion, usually for a few, more rarely for more than 10 months, lowering the risk of an acute onset of the disease and the development of late diabetic complications. However most patients lose the ability to produce insulin in a very short time [4]. The residual insulin secretion is the target for many experimental therapies aiming at preserving it as long as possible.

Insulin secretion in type 1 diabetes

The period of the preserved insulin secretion resulting in low (below 0.3 U/kg b.w.) insulin requirement or insulin-independence while maintaining normoglycaemia can be seen in the preclinical phase of T1DM (asymptomatic autoimmunization leading to a progressive loss of beta-cell mass and function) or in the so-called „honeymoon" period occurring after the onset of clinically overt hyperglycaemia and the initiation of the insulin therapy [3,5]. In most patients with a newly diagnosed T1DM the function of beta-cells is partially preserved. *Post mortem* studies in patients with a long history of T1DM suggest that such residual insulin production can be maintained even for several decades [6]. However, being minimal, it does not contribute to altered levels of C-peptide in the blood or to metabolic control. It is estimated that a transient decrease in insulin demand [7] is present in nearly 80% of children with a newly diagnosed T1DM and insulin therapy started. That dwindling demand, sometimes leading to full remission, differs between patients and depends on the baseline biochemical conditions, individual predispositions and the initial insulin regime. A flowchart of T1DM phases and corresponding insulin secretion is shown in Fig. 1.

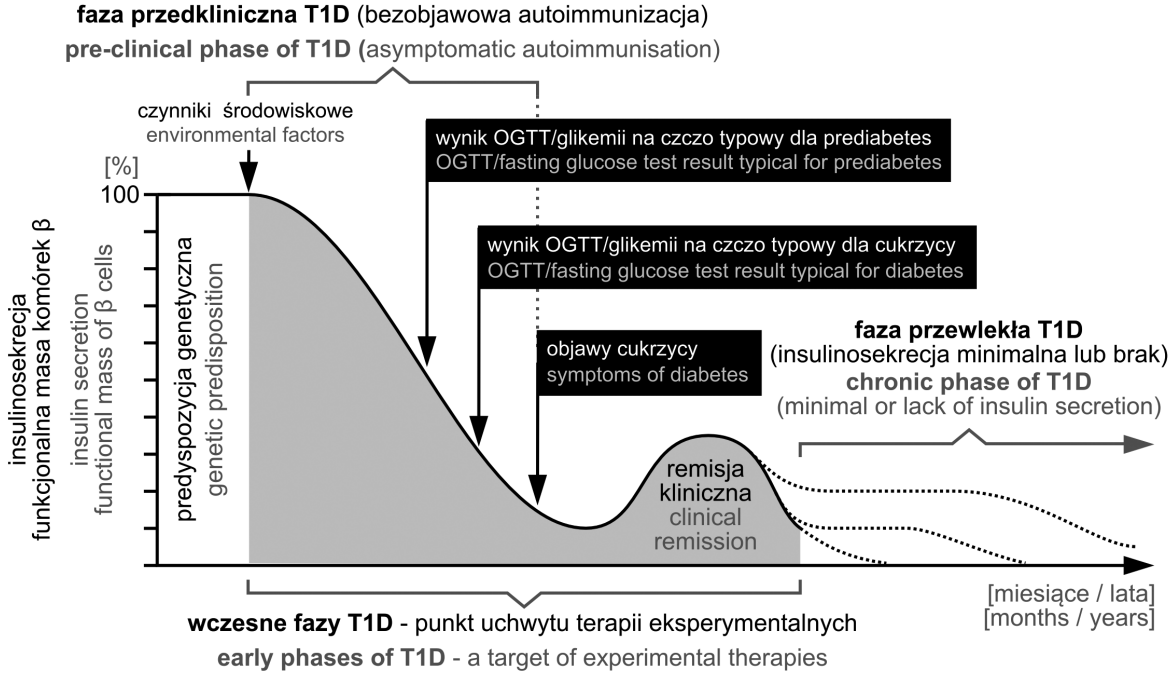

Fig. 1. Phases of type 1 diabetes (T1DM) determined by insulin secretion (OGTT – oral glucose tolerance test)
Ryc. 1. *Fazy cukrzycy typu 1 (T1D) wynikające z insulinosekrecji (OGTT – test doustnego obciążenia glukozą)*

T1DM remission can be complete (when the patient does not require exogenous insulin at all) or partial, when good metabolic control is achieved with low insulin doses (according to different definitions – below 0.5 U/kg b.w. or below 0.3 U/kg b.w.) [5,8]. However, there is much evidence that a complete withdrawal of insulin provides no benefits and results in sooner draining of the endogenous insulin reserves while low doses of subcutaneous insulin provide protection [9]. Early insulin therapy attenuates toxic effects of chronic hyperglycaemia on pancreatic beta-cells and alleviates oxidative stress, which both lead to beta-cell apoptosis [10]. This helps reduce acute inflammatory reactions within the islets of Langerhans and induces remission. During the remission phase relatively stable glucose levels can be achieved because of transient recovery of the islet secretory function and improvement in insulin sensitivity in the target tissues. As glucotoxicity decreases, the glucose uptake in the liver increases, while gluconegogenesis and glycogenolysis both decrease, leading to even better glycaemic control, lower glycated haemoglobin levels and lower demand for insulin. Higher levels of pro-insulin and C-peptide and lower levels of glucagon and GLP-1 can be seen in patients in the remission period [11]. Wegner et al. suggested classification of T1DM children into two categories: 1) those with preserved residual insulin secretion, who show slow pancreatic autodestruction, lower glycated haemoglobin levels and better metabolic control and 2) those with no residual insulin secretion. However insulin demand in both groups occurred to be similar [4].

Definitions of remission

According to the ISPAD (the International Society of Paediatric and Adolescent Diabetes) 2014 consensus guidelines, the phase of partial remission can be defined as insulin demand lower than 0.5 U/kg body weight/24 hours with glycated haemoglobin level <7% [7]. Many researchers have adopted for the diagnosis of remission the glycated haemoglobin threshold of 6% to 7.5%, or even 8% and the insulin demand <0.3 U/kg body weight/24 hours, depending on the study population [5,8,12]. In some studies C-peptide levels were also used [5]. A more specific indicator of remission might be the recently proposed insulin daily dose (DDI)-adjusted glycated haemoglobin index (HbA1c): HbA1c (%) + 4x DDI (U/kg body weight/24 h) [3,13,14]. The partial remission phase occurs within days or weeks after the introduction of the insulin therapy and can last for several weeks to several months [7]. It is usually most pronounced at 3 months of the initial diagnosis of T1DM [5], and afterwards the requirement for insulin is gradually increasing. The incidence of remission is highly variable, and in different reports ranges from just a few to 90% of all T1DM patients [12], posing even greater difficulties in defining the remission itself, estimating its frequency and timespan.

C-peptide

Measurements of C-peptide serum levels, fasting and dynamic (after stimulation with intravenous glucagon or an oral standardised liquid meal), are used to estimate pancreatic beta-cell endocrine function. C-peptide is a part of pro-insulin molecule and is secreted into the bloodstream together with insulin in equal molal amounts. Being more stable and having a longer half-life makes it a good marker of an endogenous insulin production. Reference values differ between laboratories and depend on the assay. According to the DCCT (Diabetes Control and Complications Trial) results, fasting C-peptide level above 0.23 ng/mL [9] means the insulin secretion is preserved. However it does not mean that values >0.23 ng/mL, but lower than the lower limit of normal fulfil the body requirements in terms of proper glucose metabolism. The physiological role of C-peptide is being discussed as it exhibits its own biological activity through direct paracrine and endocrine actions on a hypothetical G-protein-coupled receptor and activation of MAP-kinases as well as eNOS pathway, probably having its own – independent of insulin – role in protecting a patient from diabetic complications [4].

Predictors of remission

Chances for remission are higher in patients with **higher C-peptide concentrations, lower HbA1C levels** and **no ketoacidosis at the time of initial diagnosis of diabetes** [14]. The longer the period of hyperglycaemic symptoms and the more pronounced the acid-base disturbances – the smaller is the chance for remission [12]. That is why **early diagnosis** of diabetes is so important. Other factors that affect the occurrence and duration of remission are (among others): **age at diagnosis, genetic predispositions, severity of autoimmune processes, body weight, treatment regimen and lifestyle.** There are no consistent data on the effect of sex on remission [3,5,13–16].

Usually insulin production is decreased in patients diagnosed at a younger age or with high levels of ICA, as a result of an increased beta-cells' destruction. Numerous studies have shown that lower chances for remission are in youngest children (<5 years of age) and adolescents (>12 years of age). More aggressive disease course in the youngest age group may be linked with lower C-peptide levels, while in adolescents, it can stem from physiological insulin resistance leading to an increased insulin demand [7,15]. That is why diabetes usually runs a milder course in patients after puberty, including young adults with LADA diabetes, and their chances for remission are greater. A study by Chase et al., done on a group of 552 children with a newly diagnosed T1DM, has shown that older patients had longer duration of remission as compared to younger ones (<5 years of age), and their remission could be maintained more effectively, especially if therapeutic interventions were introduced early in the disease course [10].

Another prognostic factor seems to be a lack of significant weight loss before the diagnosis, which might indirectly suggest lower catabolic activity [16]. As for genetic factors, inheritance and carrier status, subjects lacking HLA-DR3 and -DR4 usually exhibit faster loss of their beta cell mass [3].

Optimal choice of therapy is of utmost importance for the disease course. The DCCT study has proven that an **intensive functional insulin therapy** (with insulin analogues) can maintain endogenous insulin production, decrease requirement for

insulin and improve metabolic control versus the conventional therapy. This, in turn, can reduce the risk of late vascular complications of diabetes, such as retinopathy and nephropathy, as well as severe hypoglycaemic episodes, although the best outcomes can be achieved when intensive insulin therapy is started within the first three months of the initial diagnosis [9]. Niedźwiedzki in his studies of young adult patients with T1DM has shown that the remission occurrence itself, independently of its duration, can **reduce the risk of microangiopathic complications**. In this population of patients partial remission could be achieved in 70% of subjects, provided their treatment had been started with **intensive functional insulin therapy** [5]. As the effect of the insulin therapy on beta-cell function is transient [17], it is important to further improve the methods of treatment and self-control of diabetes, also by means of **electronic devices** improving glycaemic control (CGM, continuous glucose monitoring) and insulin administration (CSII, continuous subcutaneous insulin infusion) by insulin pump.

Benefits of a **healthy lifestyle** are also very important. A balanced diet with low glycaemic index as well as regular physical activity help to control glycaemia and decrease insulin demand. Physical exercise, including sports training, increases insulin sensitivity in tissues and has anti-inflammatory properties [5,16]. Smoking has been proven to reduce the chances for achieving remission and to shorten the duration of the remission by decreasing insulin secretion and increasing insulin resistance [18]. Moreover, smoking can induce the production of free radicals and proinflammatory cytokines, which can increase the risk of diabetic complications. Higher levels of **proinflammatory cytokines**, including IFN-gamma, contribute to lower C-peptide levels and faster progression of the disease [11].

What is of key importance for maintaining normoglycaemia is also a good control of hyperglycaemic factors, such as infections or conditions accompanied by stress hormone output. The effect of blood glucose levels in early diabetes on long-term metabolic control and development of complications is related to the so-called metabolic memory programming in cells [5,19].

As epidemiological studies indicate a correlation between an increased risk of T1DM and **vitamin D** insufficiency, appropriate supplementation and prevention of hypovitaminosis may also play a role in supporting insulin secretion, because calcitriol affects insulin activity by modulating the expression of VDR genes, localized in the promoter region of an insulin-coding gene [20]. Calcitriol levels show positive correlation with insulin sensitivity, while vitamin D insufficiency inhibits beta-cell function [21]. The immunomodulatory effect of cholecalciferol, resulting from the presence of its receptor (VDR) in most cells of the immune system (especially antigen-presenting cells and activated T lymphocytes), is important for the development of tolerance and anergy in the autoaggressive environment. Administration of vitamin D to NOD mice (an animal model of T1DM) has been shown to alleviate inflammation of the pancreatic islets and decrease the incidence of diabetes [22].

New treatment hopes for improving and sustaining insulin secretion

According to the most recent ISPAD (International Society of Paediatric and Adolescent Diabetes) guidelines, until now no therapy has been proven to be effective in preventing T1DM and any interventions, both in preclinical phase (primary and secondary prevention) and after diagnosis of the disease (tertiary prevention), are allowed only as a part of clinical trials with appropriate protocols [7]. The most recent knowledge of the mechanisms underlying T1DM has resulted in a number of new therapeutic concepts with the aim to stop or even reverse beta-cell autoimmune destruction at an early stage and to induce immune tolerance. Such **tolerance** may be achieved by inducing anergy in the disease-causing effector T-lymphocytes, by deleting the said lymphocytes or by inducing the regulatory T-lymphocytes (Tregs).

Cellular therapies with patients' own, artificially multiplicated regulatory T-lymphocytes (Tregs) and umbilical cord blood stem cells have been well tolerated and safely used in children. The therapy with **regulatory T-lymphocytes (Tregs)** consists in isolating Tregs from patient's own blood sample (about 250 ml), multiplying them in vitro and transfusing such load back after 10-14 days. Repeating the procedure after 3 months helps maintain the treatment effect. In the Tregs therapy group insulin secretion was maintained for a longer period of time and considerably higher C-peptide levels were observed versus the control group. No serious adverse effects have been reported after using Tregs in paediatric patients [23]. At present, there is a **TregVac2.0.** clinical trial conducted in Poland, combining Tregs with rituximab (an anti-CD20 monoclonal antibody).

There has been much hope about therapies with umbilical cord blood, which is a rich source of Tregs and multipotent stem cells (able to differentiate into beta cells). Although in animal models of diabetes the blood glucose lowering effect has been observed, in children with T1DM no benefits have been proven with autologous umbilical cord blood transfusion [24]. Promising results have been obtained in phase I/II trials conducted in China and Spain, implementing cord blood properties in the **Stem Cell Educator system**, also known as the „artificial thymus". The patient's blood there flows in a closed unit through a cell separator and a device with unique CB-SC stem cells attached; these CB-SC form short-term colonies with patient's lymphocytes acting as immune programmers inducing immune tolerance. The advantage is that patients do not receive allogeneic stem cells, which ensures safety of the therapy. These studies may lead to regeneration of beta-cells, increased insulin secretion and an improved metabolic control in patients with T1DM already in progress. Moreover, the method gives hope for reversing autoimmune processes in other autoaggression diseases [25]. Immunomodulatory therapies alone, using **biological drugs** such as abatacept (immunoglobulin-coupled CTLA-4 antigen) or teplizumab (monoclonal anti-CD3 antibody), that inhibit a full-blown reaction of T-lymphocytes, as well as rituximab (monoclonal anti- CD20 antibody decreasing the number of B-lymphocytes presenting pancreatic islets'

antigens), allowed only for temporary maintenance of beta-cell function in patients with a newly diagnosed T1DM [2]. Similarly, the therapy with granulocyte-colony-stimulating factor (**G-CSF**) did not provide an expected rise in Tregs and C-peptide levels in patients with a newly diagnosed T1DM. The authors stress the need for combined therapies that cause not only stimulation of selected immune cell lines but also the depletion of others [26]. As most of the monotherapies in use bring no lasting effects, the latest scientific reports expect an improvement in treatment approaches to the earliest stages of T1DM by combining the already known therapies to create synergy [2]. Some say immunosuppressive drugs, such as rituximab or teplizumab, should be combined with etanercept (a TNF-alpha inhibitor) or abatacept (CTLA4-Ig) at an early stage of the disease. Expected effects could be then maintained by auto-antigens (GAD, pro-insulin) or beta-cell regeneration-enhancing factors, such as GLP-1 (glucagon-like peptide 1) [27].

Inhibition of the excess activity of the immune system in the initial phase of T1DM could also be achieved by immune ablation and subsequent **AHSCT** (autologous hematopoietic stem cell transplantation). In the study of 24 adult patients who underwent ablation with cyclophosphamide and ATG (antithymocyte globulin), mean duration of the remission after the AHSCT in 20 out of the 24 patients was 31 months (ranging from 9.5 to 80 months). Also, HbA1C levels in this group were satisfactory. Apart from 1 death resulting from neutropenic sepsis no serious adverse effects were reported. The therapy is not devoid of adverse event risk, but for some patients it offers the chance for more than 5 years of insulin-independence [28].

Although methods of **immune tolerance** induction include an oral administration of protein antigens, up until now interventions of this kind have been ineffective because of an extensive protein degradation in the alimentary tract.

Numerous studies yielding positive results are being conducted **in animals and in vitro**. Robert et al. have reported the effectiveness of a combined, antigen-specific therapy (combiGAD) in inducing tolerance in NOD mice by using *Lactococcus lactis* (LL) programmed to secrete GAD65 antigen and interleukin-10 in the gut, obtaining a decrease in the number (but not total elimination) of effector T-lymphocytes together with an increase in the number of Tregs (Foxp3+CD4+CD25+). The intervention reduced hyperglycaemia, induced remission and protected against *insulitis* progression. These preclinical data suggest a large potential of autoantigen-secreting LLs in inducing tolerance in the course of T1DM [29]. Another antigen-specific therapy tested on NOD mice is the combination of intraperitoneal administration of *Litomosoides sigmodontis* (rodent gut parasites) and intranasal proinsulin. The authors report that the therapy prevented the onset of overt diabetes by alleviating the inflammatory process in the pancreatic islets and by increasing the number of Tregs in the pancreatic lymph nodes [30]. There have also been reports of a system of micromolecules containing immunosuppressive factors and antigens (vitamin D3, a 9-23 peptide being a fragment of the insulin molecule, TGF-β1 and GM-CSF), administered subcutaneously as a „vaccine" promoting immune tolerance through dendritic cells developed; 40% of the „vaccinated" mice were protected against T1DM [31].

Other studies on rats with streptozocin-induced T1DM have shown that it is possible to limit the oxidative stress and apoptosis of the pancreatic beta-cells by using DJC (Danzhi Jiangtang Capsule), based on a Chinese formula and used earlier for the treatment of diabetes [32].

However a multicenter scientific consortium, having analysed earlier prospective preclinical studies, has not proven superiority of the combined anti-CD3 and IL-10 blockade therapy versus the anti-CD3 therapy alone in murine model. These results support the importance of large, multicenter preclinical trials in verifying treatments and setting therapeutic priorities for future clinical usage in humans [33]. Therapeutic interventions in the animal model of T1DM are very promising, however their correspondence with results in humans is still not clearly confirmed.

Moreover, there are ongoing studies to investigate the effectiveness of genetically modified human beta-cell lines that could be used for an in vitro assessment of the immune response variants contributing to the T1DM pathogenesis and occurring after beta-cell transplants. This could enable a preclinical evaluation of emerging interventional strategies aiming at protecting beta-cells from immune destruction [34].

Summary and conclusions

Preservation of residual insulin secretion does not always suggest the remission in T1DM. It can be detected in a much larger population of patients who do not meet the currently accepted criteria of remission, mostly because of too high demand for insulin. This value is largely dependent on the quantity and quality of the ingested food, physical activity and hormone balance. The period of intensive growth and puberty is widely known to be accompanied by higher insulin demand, mainly because of increased insulin resistance and increased appetite. A large subset of patients requires relatively low doses of insulin per kilogram of body weight, but it does not necessarily mean that they secrete enough insulin to be in remission; the confounding factors being low carbohydrate intake as compared to protein and fat, low appetite or very high physical activity. The same applies to the youngest children, <5 years of age, in whom C-peptide levels are usually low. Therefore, in terms of protection against complications it seems more important to apply an objective indicator of preserved insulin secretion, such as C-peptide level in the serum, rather than to rely on remission criteria such as insulin demand and metabolic control. Patients and their caretakers must be informed that remission is only temporary and does not mean the patient is cured, and that at present there is no treatment available to restore beta-cells function in the long term [7]. The extensive knowledge of factors and mechanisms underlying the onset and progression of diabetes in its early phase gives us hope for new therapeutic interventions that could delay the progression of the disease. However, at the moment there is no treatment

that would cure T1DM, and the approved therapies still rely on insulin substitution and promoting healthy lifestyle. Optimal treatment approaches should aim at restoration of immune homeostasis, preservation and regeneration of the remaining beta-cells and their protection from further damage. Studies show that the most effective inhibition of beta cell destruction can be achieved by multi-target combination therapies [2, 27]. The main problem about immunomodulatory therapies is that they are prescribed too late in the course of the disease, when the autoimmune process is too advanced for them to have any meaningful effect. In part it is due to the delayed diagnosis of the disease. Moreover, because of the risk of adverse effects, only few therapeutic programmes are allowed to be used in paediatric populations, while T1DM is diagnosed mostly in this group of patients. The above-cited publications confirm that early diagnosis along with early therapeutic interventions with both pharmacological and non-pharmacological treatments have the potential to prevent hyperglycemia and slow down the process of beta-cell destruction. An extended phase of residual insulin production together with appropriate self-control of diabetes can improve metabolic control and reduce the risk of diabetic complications that remain to be a big challenge for diabetology.

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