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REVIEW

Crosstalk Between Immunity System Cells and Pancreas. Transformation of Stem Cells Used in the 3D Bioprinting Process as a Personalized Treatment Method for Type 1 Diabetes

Paulina Niedźwiedzka-Rystwej[1] · Mikołaj Wołącewicz[2] · Piotr Cywoniuk[3] · Marta Klak[3] · Michał Wszoła[3]

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Abstract
Interactions between the immune system and the pancreas are pivotal in understanding how and why β cells' damage causes problems with pancreas functioning. Pancreatic islets are crucial in maintaining glucose homeostasis in organs, tissue and cells. Autoimmune aggression towards pancreatic islets, mainly β cells, leads to type 1 diabetes—one of the most prevalent autoimmune disease in the world, being a worldwide risk to health of many people. In this review, we highlight the role of immune cells and its influence in the development of autoimmunity in Langerhans islets. Moreover, we discuss the impact of the immunological factors on future understanding possible recurrence of autoimmunity on 3D-bioprinted bionic pancreas.

Keywords Type 1 diabetes · Immune cells · Pancreas · Bioprintig · CRISP/CAS9

Abbreviations

APC	Antigen-presenting cells
CITx	Clinical islets transplantation
DCs	Dendritic cells
T1D	Type I diabetes

Introduction

Year-to-year, a number of people suffering from diabetes mellitus, a disorder affecting glucose metabolism, increase, reaching ~ 422 million worldwide (https://www.who.int/news-room/fact-sheets/detail/diabetes). It is estimated that by the end of 2030, a group of diabetics will be doubled. The incidence of this disease among the population is constantly increasing, mainly in highly developed countries, since the 1950s, which allows to classify type I diabetes (T1D) as a civilization disease (Todd 2010; Van Belle et al. 2011). The most common form of diabetes is type II diabetes, which can result from poor dietary habits, insufficient physical activity, and increased body mass index (Cooke 2009; Olokoba et al. 2012). Less common, although also increasing, is T1D which is autoimmune disease attacking pancreatic Langerhans islets (Oras et al. 2019). The islets are composed of several types of cells: α, β, δ, ε and pancreatic polypeptide (PP). Each type plays different role in secretory function of pancreas and, among others, α and β cells produce glucagon and insulin, respectively (Bluestone et al. 2010). Interplay between these two compounds provides proper glucose level administration in blood. In T1D, organism's immune system, for still not entirely understood reasons (it is thought that they may be genetic predisposition and unspecified environmental factors), attacks β cells leading to blood glucose control disorders, which can lead to both acute conditions (ketoacidosis) and secondary complications (heart disease, blindness) (Bluestone et al. 2010). It has been already shown that autoaggression in T1D starts in mutations in highly polymorphic region of genome encoding proteins of major histocompatibility complex (MHC) responsible for antigen presentation to immune cells (Bluestone et al. 2010; Buch et al. 2006; Todd 2010). Due to its origins, it is very complicated to identify particular mutation or group of mutations causing aberrant recognition of β cells by immune system. First studies on immunological background of T1D reach 1980s and, so far a repertoire of immune cells interacting with Langerhans islets emerges (Fig. 1): (1) neutrophils and

✉ Marta Klak
marta.klak@fundacjabirn.pl

[1] Institute of Biology, University of Szczecin, Szczecin, Poland

[2] Students' Scientific Immunology Circle at the University of Szczecin, Szczecin, Poland

[3] Foundation of Research and Science Development, Warsaw, Poland

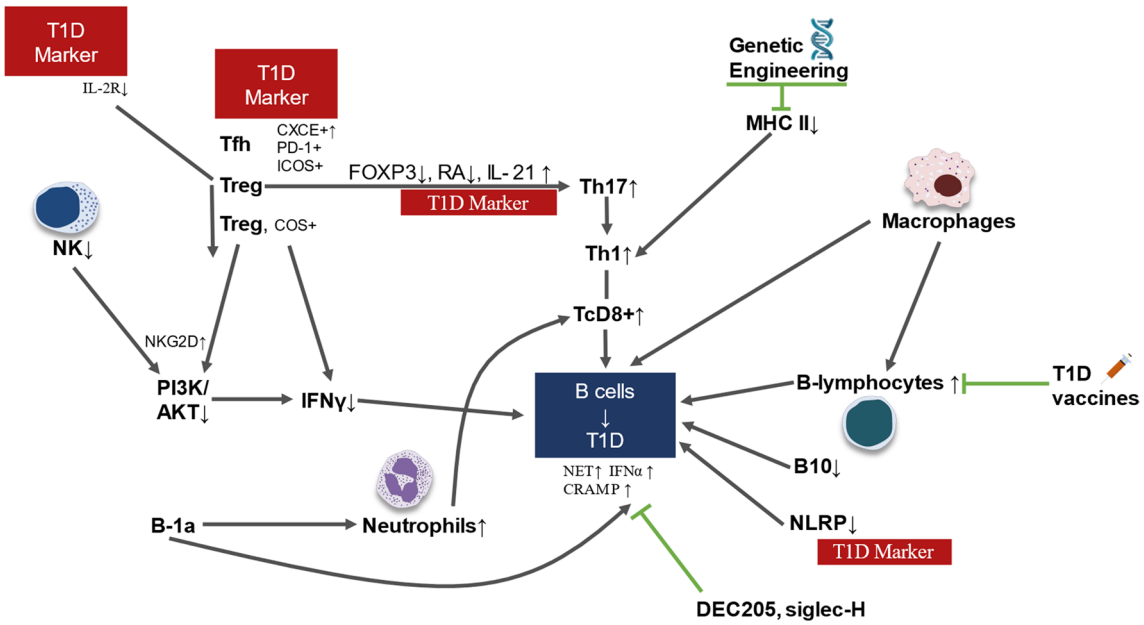

Fig. 1 Interactions between immunological cells in T1D and its possible impact on future T1D treatment. *NET* neutrophil extracellular trap, *RA* retinoic acid, *NLRP* NLR family pyrin domain containing

macrophages; (2) NK cells; (3) T lymphocytes and (4) B lymphocytes indicating complexity of this crosstalk (Diana et al. 2013; Fraker and Bayer 2016; Oras et al. 2019). Currently, intensive works are ongoing on development of new T1D treatment approach fusing biotechnology, advanced technology and transplantology. In view of permanent shortage of organs to transplant and insufficiency of insulin pumps, an idea of 3D printable bionic pancreas composed of genetically engineered differentiated stem cells has arisen (Ravnic et al. 2017). This approach might enable to generate unlimited access to organs and accelerate donor-independent transplantation strategy. However, to achieve this goal, a deep understanding of immune mechanisms standing behind pancreatic autoaggression seems inevitable. In this review, we report current knowledge about particular immune cell types and the mechanisms of their aberrant interactions with pancreas in T1D. We also highlight efforts to develop hypoimmunogeneic stem cells which would be applied to 3D print of bionic pancreas.

Neutrophils and Macrophages as Innate Immunity Elements in T1D

Research on neutrophils and their effect in the onset and progression of T1D has increased in recent years (Amulic et al. 2012; Huang et al. 2016; Nathan 2006). Exciting discoveries have provided new insights of neutrophils in the initiation of the disease, which found that neutrophils can induce T1D through infiltrating into the pancreatic islets and interplaying with other immune cells (Nathan 2006). Other studies have also shown that T1D patients have higher leukocyte, monocyte and neutrophil levels, and elevated hs-C-reactive protein, interleukin (IL)-6 and IL-10 levels compared to controls (Karavanaki et al. 2012; Oras et al. 2019; Xu et al. 2013). It has been shown that almost every function of neutrophils is affected by T1D—from chemotaxis to phagocytosis and apoptosis, where upregulation, downregulation and no changes also have been observed (Diana et al. 2013; Lande et al. 2011).

There are also studies available in NOD mice showing the role of neutrophils in the pathogenesis of T1D (Diana et al. 2013). The histological analysis has confirmed the abundance of cathelicidin-related antimicrobial peptide (CRAMP) secreting neutrophils (Fig. 1). Studies have shown that pancreatic neutrophils released neutrophil extracellular traps containing DNA, and were associated with CRAMP peptides and caused the activation of interferon (IFN)-α-secreting plasmacytoid dendritic cells (pDCs) (Lande et al. 2011; Diana et al. 2013).

Researchers concluded that the reduction of neutrophils in young mice reduced the amount of IFN-α secreting pDCs in the islets of treated mice. In this way, T-cell responses were suppressed in older mice. Further studies also showed that in NOD mice with complex immunodeficiency, CRAMP-producing neutrophils are activated by IgG-secreting B-1a (FcγR receptor) (Diana et al. 2013).

Researchers also concluded that IgG-secreting B-1a lymphocytes and CRAMP-secreting neutrophils work together to activate pDC to produce IFN-α (Diana et al. 2013).

However, new and detailed research is required to determine how neutrophils are activated and recruited to damage the pancreatic islets by toxic substances derived from neutrophils or by interacting with other cells. A better understanding of the role of neutrophils can help to diagnose, treat and maybe even prevent T1D. Unanswered questions may be encouraging for further research on this very topic. However, new genetic and antibody-based tools are needed to correctly determine the role of neutrophils in vivo (Mantovani et al. 2011).

It has been suggested that macrophages play an important role in the pathogenesis of autoimmune T1D (Yoon et al. 1998). Unfortunately, there are only a few studies that show the role of macrophages on the immunopathogenesis of T1D in human patients. However, studies in diabetes mouse model show that macrophages are necessary for the development of cytotoxic T lymphocytes that cause the destruction of pancreatic β islets (Jun et al. 1999; Zapata et al. 2014).

Although the role of macrophages in T1D remains certain, the exact mechanism of actions is still unknown. Macrophages play an important role as antigen-presenting cells (APCs) and actively participate in triggering inflammatory responses by activation of B and T lymphocytes which initiate insulitis (Zapata et al. 2014).

The importance of research on this particular type of cells has increased in recent years. Now, macrophages are considered as promising targets for the development of immunotherapy in the T1D (Fig. 1).

The Role of NK Cells in Pancreas Immunity

In patients with T1D, significant differences were found in the number and percentage of NK cells (including a reduced percentage in NK cell subgroups $CD56^{dim}CD16^{+}$ and NK $CD56^{dim}CD16^{-}$) and their subsets (Oras et al. 2019; Takahashi et al. 2007).

A significant reduction in the number of NK cells and their subsets ($CD56^{br}CD16^{-}$, $CD56^{dim}CD16^{+}$, $CD56^{dim}CD16^{-}$) has also been confirmed by studies of two other research groups (Qin et al. 2011; Zhang et al. 2017). One possible explanation for the lower NK number in T1D patients in relation to healthy individuals may be associated with a reduced response to IL-2 and IL-15 interleukin stimulation (Qin et al. 2011), which, when combined with impaired number of cells in the NK cell compartment, may result in increased susceptibility for some viruses (e.g., enterovirus) (Fraker and Bayer 2016).

In addition, analysis of NK cells before and after IL-2 stimulation showed (Qin et al. 2011) that, unlike healthy people, TD1 patients did not inhibit NKG2D ligand. These cells also showed reduced NKG2D-dependent cytotoxicity and reduced IFN-γ secretion. Finally, NK cells from patients showed defects in NKG2D-mediated activation of the phosphoinositide 3-kinase–AKT pathway (Fig. 1).

APCs and T1D

APCs are critical for exposition of peptide markers to T cells resulting in immunological reactions that may lead to apoptosis of β cells (Boldison and Wong 2016). Moreover, β cells transfer secretory granules to resident islet APCs and this allows the recognition of β-cell antigens by T $CD4^{+}$, which is crucial to further immune responses (Boldison and Wong 2016). The pancreatic islets contain mainly $CD11c^{+}CD11b^{+}F4/80^{+}CD80^{+}$, expressing MHC class II (MHC II), but also subpopulation expressing CD103 ($CD11c^{+}$MHC II^{+}) has been identified (Boldison and Wong 2016).

The problem observed in studies concerning pancreas, mainly in the course of T1D, is the reduced frequency of DCs, including reduced levels of different subsets: pDC, myeloid DC (mDC), slan-CD16-mDC and slan-$CD16^{+}$ mDC in patients with T1D compared to controls (Oras et al. 2019).

Several research groups have shown that some DC cell surface molecules (such as DEC205 and Siglec-H, among others) can be used to direct specific subsets of DC cells to generate immune tolerance (Fraker and Bayer 2016; Lee et al. 2013; Oras et al. 2019; Takahashi et al. 2007). It was shown (Qin et al. 2011) that targeting autoantigen myelin oligodendrocyte glycoprotein to pDC via Siglec-H molecule may result in T helper (Th) cell and antibody responses inhibition in an antigen-specific manner. The study demonstrated the potential use of DC cells to inhibit antigen-specific immune responses and inhibition of Th-dependent autoimmunity, such as T1D (Fig. 1).

Vaccination with antigens specific for pancreatic islets is also a possibility. Clinical studies with DiaPep277, GAD and oral insulin have shown minimal effect of these substances on T1D prevention and did not reduce the required insulin dose. Nevertheless, they provide minimal benefits such as stopping the complete destruction of β cells and the increase in C peptide levels in some patients. This suggests they still should be considered as T1D treatment strategies. However, the use of antigen-specific vaccinations may delay the occurrence of diabetes-related complications (Lee et al. 2013; Qin et al. 2011; Zhang et al. 2017).

Therefore, DC-dependent biological tolerance mechanisms require further investigation. Early therapy is especially important because treatment after T1D symptoms is more difficult because natural homeostasis must be restored. In addition, after the onset of diabetes, surgical replacement of damaged β cells and the creation of an effective method of immunosuppression would be necessary.

T Lymphocytes are the Key Cells in Moderating Pancreas Reactions

The autoimmune destruction of pancreatic islet β cells is caused by the breakdown of the immunological tolerance, involving the activation of Th1 and Th2 pathways (Bluestone et al. 2010). Due to the lack of MHC II molecules on β cells, autoantigens are released from the pancreas followed by the transport to the draining lymph nodes by DCs (Yadav et al. 2004). This leads to T cells priming, expansion and recruitment to pancreatic islets, causing the destruction of β cells, impairment of insulin production and development of T1D (Yadav et al. 2004).

It was shown that IFN-γ and IL-2 are the key mediators of B-cell autoreactivity; whereas, IL-4, IL5, and IL-13 have a protective effect against autoimmunity in T1D (Shao et al. 2012).

The role of T $CD8^+$ cells has also been emphasized in the pathogenesis of T1D, showing that the level of these cells in peripheral blood of the patients with T1D dominantly increases in the course of disease (Di Lorenzo et al. 2007). Moreover, T $CD8^+$ lymphocytes are predominant subset in pancreatic cancer (Ademmer et al. 1998).

An important subpopulation of T lymphocytes is T regulatory (Treg) cells ($CD4^+CD25^+$) with the ability to suppress effector T-cell responses, as the dysregulation of those cells leads to autoimmunity and several different homeostasis disorders (Boldison and Wong 2016). Studies revealed that pancreatic Tregs have a different profile in comparison to Tregs in spleen, including differential expression of suppressive mediators (IL-10, Fgl1, Lag3) and chemokine receptors (CXCR3, CCR5) (Lu et al. 2017). Moreover, another proof of a tissue-specialized transcriptome of pancreatic Tregs is the difference in expression of genes involved in cell growth and proliferation. Genes encoding some of the functional molecules, such as glucocorticoid-induced TNF receptor (*GITR*), *CD103*, *Nrp-1*, *IL-10*, *CTLA-4* are overexpressed, while genes encoding transcription and signaling factors, like *OBF-1*, *Tcf7* and *Smad1* are downregulated (Lu et al. 2017). It is known that IL-2 regulates FOXP3 expression—a hallmark of Tregs, and the defect in IL-2R signaling in T1D leads to impaired function of Tregs (Long et al. 2010). These findings suggest a novel treatment for T1D on the basis that elevated IL-2R signaling may be a biomarker of this disease (Long et al. 2010). Interestingly, it was also shown that in pancreatic islets, Tregs not only inhibit CD4 T-cell function, but also impact the role of T $CD8^+$ cells—an important subpopulation of T cells in pancreas (Mahne et al. 2015). It has been documented that fewer level of $CD8^+$ T cells is the result of reduced chemokine recruitment and IFN-γ production via mTor signaling (Mahne et al. 2015).

A very interesting fact concerning Tregs in pancreas is the existence of a unique subpopulation—$ICOS^+$ Tregs—a subset expressing ICOS—a CD28 superfamily related molecule involved in T-cell activation and survival (Herman et al. 2004). This subset constitutes around 8% of $CD4^+$ and 50% of $FOXP3^+$ Tregs and ICOS expression is known to be extremely reduced in NOD mice, suggesting that ICOS might play a protective role in T1D (Herman et al. 2004). The $ICOS^+$ Tregs exhibit a great proliferative capacity and are more effective at effector T cells (Teffs) suppressing (Kornete et al. 2012). Also, due to a similar activation status with $ICOS^-$ Tregs, pancreatic $ICOS^+$ Tregs may serve as a memory Tregs in islets (Kornete et al. 2012). Moreover, $ICOS^+$ Tregs express IFN-γ at higher levels, leading to prevention of IFN-γ production and due to this, $insulin^+$ β cells expressing IFN-γ could promote apoptosis of islet cells. Thus, Tregs may have an indirect role to prevent the destruction of the islets (Kornete et al. 2015).

Another abundant subpopulation of Tregs in pancreas is $CXCR3^+$ Treg, recognized as preventing inflammation subset (Tan et al. 2016). Authors are suggesting that an unusual property of this subpopulation is their capacity to control sex bias of T1D incidence in NOD mice (Tan et al. 2016).

Moreover, a subset of T follicular helper (Tfh) cells was found in children diagnosed with T1D (Heuts et al. 2017). The significantly increased level of $CXCR5^+PD\text{-}1^+ICOS^+$ cells may be also a new marker of T1D risk, also due to the fact that in patients with T1D, overproduction of IL-21 is noticed and this cytokine is characteristic for Tfh (Heuts et al. 2017).

An important observation was made by Korean researchers, leading to the conclusion that the use of Tregs should be explored in islets transplantation on the basis of the results that Tregs improve islets functions and viability via transforming growth factor β1 secretion pathway (Choi and Kim 2018). There were also studies to develop a novel approach for local immunoprotection using Tregs attached to the surface of the islets before transplantation and it was shown that local delivery of immunoprotective Tregs may be an alternative to currently used immunosuppression (Marek et al. 2011).

Studies have also been conducted on the role of Th17 lymphocytes. It was confirmed that a high level of IL-17 is expressed in the pancreas, leading to a potential role of Th17 lymphocytes (Vukkadapu et al. 2005). Moreover, IFN-γ may also be a factor contributing to Th17-induced diabetes (Martin-Orozco et al. 2009). The inflammation of pancreas may be increased by Th17 cells but only under certain circumstances, that is conversion to Th1 cells (Martin-Orozco et al. 2009). This dependence was also partly confirmed by the study of IL-17A concentrations and variants in T1D patients (Fores et al. 2018), where it was concluded that lower expression of IL-17A receptor in $CD3^+$ and $CD4^+$

T lymphocytes impacts the effects of IL-17A in peripheral tissues; whereas, IL-17A allelic variants were not related with T1D susceptibility (Fores et al. 2018). Nevertheless, the balance between Teff and Treg is crucial to maintain the regular immune response and, as studied by several researchers, Th17 may influence the balance causing immunological pathology of T1D (Yadav et al. 2004). Autoimmune attack on the pancreatic islets is caused as a sequence of conversion from Th17 to Th1 phenotype of lymphocytes and Th17 to T $CD8^+$ lymphocytes (Ankathatti Munegowda et al. 2011). In the pathogenesis of T1D, excessive expansion of Th17 is also indirectly mediated by Tregs, via diminished expression of FOXP3, strengthening of IL-21 signalling and dysregulation of retinoic acid pathway (Shao et al. 2012).

Another important role for Th17 lymphocytes in pancreas, mainly in the course of T1D, is the ability of the cells to regulate the gut flora (Bedoya et al. 2013). It was confirmed that T1D young patients have a significantly different composition of gut microbiome comparing to healthy individuals (de Goffau et al. 2013). It was shown that *Bifidobacteria* and lactate- or butyrate-producing species not only supply butyrate-producing species with lactate and acetate but also enhance the intestinal epithelial barrier function by modulating the gut mucosa (de Goffau et al. 2013). This possibly prevents the translocation of *Bacteroides*, which were confirmed to be more highly abundant in children with β-cell autoimmunity (de Goffau et al. 2013).

Nevertheless, mainly the studies on T1D show that T lymphocytes are not the only immune system cells with a broad role in pancreas (Fig. 2).

B Lymphocytes

Studies suggest a very important role of B lymphocytes, next to T lymphocytes, in the immunopathogenesis of T1D (Deng et al. 2016). However, all possible functions of B cells in human T1D remain unclear (Battaglia 2014).

Various subsets of B cells in the peripheral blood were investigated (Deng et al. 2016). The major finding was a distinct alternation of B-cell subsets across the diabetes spectrum and their association with clinical features. Moreover, studies show the decrease of regulatory B cells—B10 frequency in all diabetes types and the lowest level was noted in T1D.

B cells are generally known for their function in humoral immune response by producing antibodies. They have also been shown to play an immunoregulatory role in the prevention of autoimmune disease by producing IL-10 and downregulating T-cell responses (Amu et al. 2010; Carter et al. 2012; Deng et al. 2016; Yanaba et al. 2008). The observed decreased level of B10 cells may have an impact on the breakdown of self-tolerance, which leads to β-cell destruction in patients with T1D (Thompson et al. 2014).

In summary, current data show that B lymphocytes play an important role in immunopathogenesis of autoimmune diabetes (Fig. 2). Currently, specific subsets that could be used for diagnostic purposes cannot be identified, but the findings strongly imply that the change of B-cell subtypes is related to the pathogenesis of autoimmune diabetes (Deng et al. 2016). Furthermore, it may be a good idea to consider the approach of B-cell-targeted therapy in T1D patients.

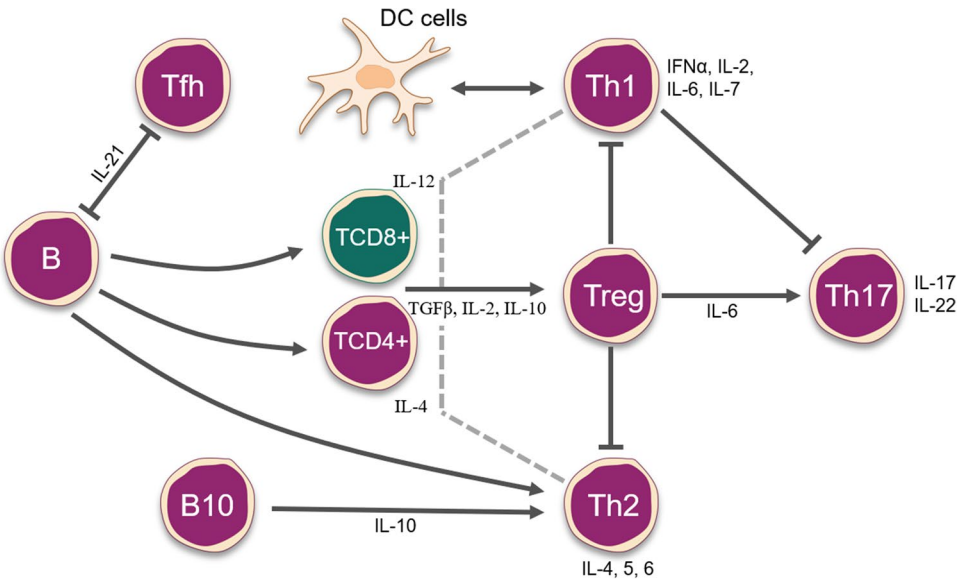

Fig. 2 Interactions between T- and B-cell subsets in T1D

Inflammasomes as a Novel Element in Understanding Immune Responses in Pancreas

Inflammasomes are complexes activated in response to infections, inflammations and autoimmune processes and their main pathway of functioning is maturation and production of IL-1β and activation of caspase-1 (Gross et al. 2011). It was already shown that IL-1β in T1D participates in the recruitment of immune cells to islets, alters insulin secretion and induces β-cell apoptosis (Lukens et al. 2011). IL-1β plays also a crucial role in cell destruction after islet transplantation (Mandrup-Poulsen 1996). There have been studies conducted where it was shown that elevated production of IL-1β, TNF-α and IFN-γ after islet transplantation induces activation of macrophages (Bottino et al. 1998). Also, studies have been conducted showing that *NLRP3* gene is overexpressed in the condition of hypoxia accompanying the T1D condition (Lebreton et al. 2018). The conclusion is very promising—possibly inflammasome genes expression might be a new marker of T1D.

Immunity of Pancreas: Future Perspectives and Impact of Bionic Pancreas Printing

The ultimate aim in T1D treatment is to reestablish proper glucose distribution disturbed mainly by the loss of insulin-producing β cells. Current medicine offers several therapeutic approaches including pump-mediated insulin supplementation, whole pancreas or clinical islets transplantation (CITx). Insulin pumps strategy is the most common; however, due to inefficient responsiveness and dosage, higher risk of hypoglycemia episodes and about 30 min of delay in glucose level in interstitial fluid measured by pumps is improper to, at least, some of the patients with T1Ds. On the other hand, T1D leads to many subsequent disorders such as nephropathy, retinopathy, cardiovascular disease (CVD) and neuropathy. These complications affect the patient's qualification to transplantation. Due to this, pancreas transplantation may be more appropriate for younger patients with fewer CVD complications while these with higher rate of CVD should rater be qualified for CITx but this procedure is at a crossroads. Since the introduction of the Edmonton protocol (Shapiro et al. 2000), improving long-term results is difficult to achieve, even for the researchers who developed the protocol (Shapiro et al. 2006). It is mainly due to islets apoptosis because of lack of extracellular matrix and lack of specific vasculature which is destroyed during the process of islets isolation. Another difficulty is an instant blood-mediated reaction observed after transplantation into a portal vein which diminishes an amount of living islets. Developing new implementation sites, such as gastric submucosa (Wszola et al. 2009, 2018a, b) led to some improvement but could not solve a problem. There is a need to develop a technique as efficient as pancreas transplantation but with a complication rate close to CITx procedure, thus, to produce an organ with extracellular matrix and vasculature around islets, but one which is not complicated like in native pancreas. Another important goal is to find a source of islets for transplantation excluding use of donors' organs, preferably with patients' own cells. It could also help to reduce or even omit the need for an immunosuppressive treatment intake. Donor-independent strategy in T1D treatment, based on human stem cells cultured in vitro and differentiated to insulin- and glucagon-producing cells (β and α cells, respectively), combined with advanced technologies such as bioprinting seems to be reasonable. Following, all required cell types (i.e., α, β or endothelial cells) could be derived from stem cells and, subsequently, incorporated into a bioink used for 3D printing of the vascularized bionic pancreas for transplantation into T1D patients. Depending on cell source, treatment should be personalized using patient's own stem cells isolated from adipose tissue or a universal, suitable for each patient, stem cell line could be developed. However, in both approaches, one of the biggest challenges is the recipient's immune barrier. In case of a personalized variant, one must overcome the primordial aspect of the disease. As T1D is an autoaggressive disorder, the immune system of a patient attacks its own β cells (Atkinson et al. 2014; Chentoufi et al. 2008; Rossini 2004). Hence, retransplantation of differentiated patient's cells carrying the same genotype will result in renewed autoaggression. On the other hand, utilizing stem cells derived from other donors will initiate the immune mechanism observed in allografts that leads to increased risk of graft rejection and requires treatment with immunosuppressant drugs for the rest of patient's life (Fleischhauer et al. 1990; Williams et al. 2017). In both cases, manipulations on molecular level seem to be a reasonable way to solve the problem with immunity. Although both, T1D-related and graft-related, immune pathways have not yet been fully understood, a number of projects investigating these mechanisms are constantly increasing. Last decades have brought us plenty of publications describing general mechanisms standing behind the immune response which, in turn, allowed for first attempts to manipulate immune machinery (Braciale 1992; Buch et al. 2006; Chang et al. 1994; Chang and Flavell 1995; Güssow et al. 1987; Russell et al. 2019; Trombetta and Mellman 2005). A milestone which accelerated such research was the discovery of prokaryotic antiviral defense system called Clustered Regularly

Interspaced Short Palindromic Repeats (CRISPR) and the development of a method based on this mechanism (CRISPR/Cas) enabling precise edition of the genome to make cells "invisible" to the immune system (Barrangou et al. 2007; Barrangou and Doudna 2016; Horvath and Barrangou 2010; Sander and Joung 2014; Xu and Qi 2019) For over 30 years, many groups have been focused on searching for master regulators of allogeneic immune response that is responsible for grafts rejection. Across the years, the number of "candidate genes" has been progressively reduced and currently, most studies are focused on silencing of *B2M* and *CIITA* genes (Buch et al. 2006; Chang and Flavell 1995; Chang et al. 1994; Gornalusse et al. 2017; Russell et al. 2019; Sliker et al. 2019; Wang et al. 2015). *B2M* (β-2-microglobulin) encodes a protein which is a structural component of all types of human leukocyte antigen (HLA) or MHC class I, respectively—protein complexes accountable for presentation of foreign antigens (i.e., pathogenic) to T cells (Braciale 1992; Güssow et al. 1987). *CIITA* gene encodes a transcription regulator of protein-coding genes for HLA (MHC) class II which stimulates indirectly B lymphocytes to produce specific antibodies (Buch et al. 2006; Chang and Flavell 1995; Chang et al. 1994; Trombetta and Mellman 2005). *B2M* and *CIITA* are, therefore, universal key players in two mechanisms of antigen presentation that allow the immune system to recognize and react properly. Recent work revealed that knock-out of these genes in transplanted cells might lead to their anonymization towards the immune system of the recipient. However, groups of Schrepfer (Deuse et al. 2019) and Cowan (Han et al. 2019) in their experiments showed that disruption of HLA class I and II alone might be insufficient. Besides antigen recognition, immune system recognizes host's own cells by the presence of particular protein markers on their surface (Deuse et al. 2019; Han et al. 2019). Lack of such signals might trigger several types of immune cells to attack. Deuse et al. (2019) presented that human-induced pluripotent stem cells (hiPSCs) with double knock-out of *B2M* and *CIITA* were recognized and attacked by murine host's immune system. An additional modification of hiPSCs' genome, in a gene coding CD47 "don't-eat-me" surface protein, caused decreased macrophage activity over the transplanted cells, which led to significantly prolonged graft maintenance (Deuse et al. 2019; Jaiswal et al. 2009). Molecular background of T1D has still not been well explained. Possibly, it is strongly connected with autoreactivity of T cells which recognize presented antigens and initiate proper cascades of the immune response (Chentoufi et al. 2008; Pugliese 2017; Rossini 2004; Spence and Tang 2016). That, in turn, presents HLA proteins as a source of autoaggression. Due to the fact that individual's HLAs repertoire is polygenic, certain combinations of HLA proteins significantly correlate with invalid autoantigen presentation (Erlich et al. 2008; Nejentsev et al.2007; Noble et al. 2010; van Lummel et al. 2012) Moreover, genes encoding HLA proteins are highly polymorphic; therefore, tracing mutations that might be responsible for aberrant antigen presentation is extremely difficult (Janeway et al. 2001) Interestingly, studies mentioned above which focused on *B2M* and *CIITA* genes' pivotal role in functionality of HLA class I and II, respectively, bring elegant solution of later potential autoaggression of recipient's immune system after hiPSCs-derived α and β cells transplantation. Silencing of *B2M* and *CIITA* should diminish all HLA protein activities, including these responsible for autoaggression; thereof, modified cells become "invisible" for T cells.

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Compliance with Ethical Standards

Conflict of interest Paulina Niedźwiedzka-Rystwej, Mikołaj Wołącewicz, Piotr Cywoniuk, Marta Klak and Michał Wszoła declare that they have no conflict of interest.

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