CellR4 2016; 4 (5): e2128

Team science in type 1 diabetes: new insights from the Network for Pancreatic Organ Donors with Diabetes (nPOD)

G. Lanzoni[1-3], M. Pokrywczynska[2-4], L. Inverardi[1-3]

[1]Diabetes Research Institute, University of Miami, Miami, FL, USA

[2]The Diabetes Research Institute Federation

[3]The Cure Alliance

[4]Department of Regenerative Medicine, Nicolaus Copernicus University in Torun, Ludwik Rydygier Medical College in Bydgoszcz, Bydgoszcz, Poland

Corresponding Author: Giacomo Lanzoni, Ph.D; email: GLanzoni@med.miami.edu

Keywords: Type 1 Diabetes, Beta cells, Autoimmunity, Team Science, Etiopathogenesis of Type 1 Diabetes, Beta cell biology, Islet autoimmunity.

Abstract

Type 1 Diabetes (T1D) is a chronic autoimmune disease leading to severe loss of insulin-producing beta cells in the pancreas. The causes of this disease are still unknown. Due to this fact, T1D cannot be effectively prevented or reversed. Pancreas or islet transplantation can revert diabetes, but require chronic immunosuppression, and both chronic rejection and recurrence of islet autoimmunity may affect long-term graft survival. The JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) is a tissue bank and a team science effort aimed at understanding the etiopathogenesis of T1D. nPOD has enabled unprecedented access to rare tissues derived from organ donors with T1D and with risk for the disease to investigators worldwide. The 8th nPOD Annual Meeting revealed new insights on T1D, covering multiple aspects of etiology, beta cell biology, and islet autoimmunity, emerging from the studies of nPOD investigators and collaborative working groups. The data being generated and shared by nPOD investigators will be instrumental in translating findings into better therapies, possibly even a cure or prevention, for T1D.

nPOD: Team science in the field of Type 1 Diabetes

Type 1 Diabetes (T1D) is a human disease characterized by an autoreactive immune attack that causes severe loss of insulin-producing beta cells in the pancreas[1]. While progress has been made in clinical trials to predict this disease[2], as of today no means exist for effectively and fully preventing or reversing T1D[3]. This is largely because the cause of T1D is still unknown[4]. Many traits of the pathogenesis and disease progression remain obscure (see Box 1 'Studying the pathogenesis of T1D'). The JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD), a tissue bank and a collaborative project, launched in 2007 to advance the understanding of human T1D through the study of the pancreas and other tissues harvested from deceased organ donors[5]. Donors recovered by nPOD include those with T1D and those with islet autoantibodies in the absence of clinically overt T1D, which could provide key insight regarding the prediabetes phase. Moreover, nPOD has recovered donors with monogenic forms of diabetes, as well as donors with Type 2 Diabetes (T2D), and those without diabetes[6]. Since its inception, nPOD has built a sizable collection of donor specimens and has supported a large number of studies led by investigators worldwide[7]. Investigators with an interest in T1D and in team science can join the nPOD research community, and can find detailed descriptions of the numerous nPOD projects on the website www.jdrfnpod.org. Moreover, nPOD is committed to promoting collaboration and team science by creating a network of nPOD investigators with an interest in the pathogenesis of human T1D who collaborate on the study of pancreata from organ donors. Thus, nPOD is a tissue repository, a network of investigators and a data sharing platform – but most of all, it is the largest and most active

team science community in the field of T1D research. Besides regular interactions via electronic and telecommunication means, nPOD investigators come together for a face to face meeting on an annual basis. The 8th Annual nPOD Meeting was held in Miami, USA, on February 21-24, 2016. The meeting was attended by over 260 scientists from all over the world. The scientific program included 25 sessions with lectures by top experts in the field, oral abstract presentations, poster presentations, discussions and working group meetings. Many important aspects of T1D were discussed – including etiology, immunology, autoimmunity, beta cell biology and regeneration. In this report we present highlights of the findings reported at the 2016 nPOD meeting, grouped by areas of interest.

BOX 1. Studying the pathogenesis of T1D

The goal of nPOD is to advance the understanding of human T1D occurrence through the study of the pancreas and other tissues from organ donors. The pathogenic events that lead to T1D remain obscure and represent the main focus of the nPOD community. When studying the disease pathogenesis it is important to take into consideration that the disease is chronic and evolves over a long period of time. Certain organ donors might have been developing T1D and the study of their organs may provide information about early stages of the disease pathogenesis and, possibly, key etiological factors. Donors who have recently developed clinical T1D are traditionally thought to be more likely to show pathological signs of active disease and to maintain a significant beta cell mass. However, data emerging from nPOD and other studies show that both autoimmunity and beta cell mass continue to be detected for several years after onset. In addition, T1D may also develop in pancreas transplant recipients, in which case the condition is termed "T1D recurrence". nPOD is making efforts to recover pancreata from organ donors in all of the above categories[6]. nPOD encourages collaborations among investigators and has implemented a data sharing platform (DataShare) to maximize the potential of the data obtained from these precious tissues by the community. The nPOD tissue bank and Pathology core provide a wealth of information for each case[6]. All working groups are committed to sharing methods and results. Here we list a series of putative 'stages' of disease, following a staging system proposed by George Eisenbarth[4], and we indicate how the work of nPOD groups could fill the gaps.

Stage 1: GENES. Over 50 risk gene variants for T1D have been identified[8,9], representing *Stage 1* of the progression toward T1D. T1D could be considered a polygenic disease with incomplete penetrance. The most important genes conferring risk are certain variants of the HLA Class II (primarily certain risk alleles in the HLA-DR and HLA-DQ loci) and HLA Class I genes, followed by a series of genes involved in immunity and beta cell function. A group of nPOD investigators (**nPOD Omics** working group) is generating and analyzing data from nPOD samples that address genetic factors with genomics, gene expression, and epigenetic approaches. Moreover, there are also coordinated efforts involving proteomic approaches. These studies have the potential to determine how the genetic and molecular bases of the disease facilitate disease progression. These studies could also enable detection of somatic mutations and other characteristics that could be associated with more aggressive forms of the disease.

Stage 2: TRIGGER. One or more environmental triggers are believed to exist and are proposed to act during *Stage 2* of the progression towards T1D[4]. The ongoing global increase in T1D incidence[3] cannot be explained by genetics alone, and it is thought to be connected to environmental triggers that promote disease development. Enteroviruses have long be suspected to play a key role in T1D but systematic studies of these and other viruses have not been possible in the absence of donor specimens. With the availability of nPOD samples, the **nPOD-Virus** working group has been formed to specifically investigate the role of viruses in T1D pathogenesis. This group is examining pancreas and other tissues from organ donors with T1D using a variety of methodological ap-

proaches to generate robust data about the prevalence of viral infections in the pancreas with T1D and their features. Hopefully, this effort will lead to the identification of the most prevalent viruses and will generate information that is critical to the development of viral vaccines and/or anti-viral therapies.

Stage 3: BETA CELL AUTOIMMUNITY. In most patients, autoantibodies targeting islet cells appear in peripheral blood[10] long before the clinical onset of diabetes, representing *Stage 3* of the progression toward T1D[4]. These autoantibodies are believed to mark the activation of the autoimmune process. The risk of disease increases when the number of autoantibodies increases[1, 10]. The beta cell mass seems to be normal, if not slightly increased, when one or two anti-islet autoantibodies are present in the blood[11]. Insulitis is the pathognomonic inflammatory lesion affecting the pancreatic islets in T1D. It consists of a lymphocytic infiltration of islets[12] even though it appears to affect only a modest percentage of islets at any given time[11]. Insulitis and beta cell loss progress with a "lobular pattern", i.e. a discrete distribution in the pancreas[4]. The lesion consists of inflammatory cells, mostly T lymphocytes, and studies of nPOD samples have shown the lesion to contain populations of autoreactive T lymphocytes reacting against islet cell autoantigens[13]. The **nPOD-Autoimmunity** working group was launched to study the features of the autoimmune responses through the study of the nPOD pancreas and lymphoid tissues (spleen, pancreatic lymph nodes), including the antigen specificity, phenotypic features and the T cell receptor repertoire of autoreactive T cells. The group also examines the role of antigen-presenting cells, including B lymphocytes, in the disease pathogenesis and is focused on revealing the mechanisms that cause an immune attack against islet cell autoantigens and on the formation of modified forms of autoantigens. In addition, nPOD has shown the feasibility of isolating islets from donors with T1D even several years after diagnosis, and investigators in this group are examining islet-infiltrating T cells. Moreover, recent studies from both experimental models and nPOD specimens illustrate the importance of extracellular matrix components in the evolution of the insulitis lesion. Some of these components are critical to islet structural integrity and beta cell function, and others are important for the trafficking of immune cells to the islets. An nPOD working group is focused on the involvement of islet and lymph node extra-cellular matrix components in the pathogenesis of T1D (**nPOD-Matrix**).

Stage 4: BETA CELL DYSFUNCTION AND LOSS. Beta cells become progressively impaired in the period that precedes the clinical onset of T1D. The decline in beta cell function is evidenced by loss of first-phase insulin response to an intravenous glucose challenge and later by the appearance of impaired glycemic regulation[14]. Severe beta cell dysfunction and loss become apparent during *Stage 4* of the progression towards T1D[4]. It is emerging, however, that the extent of beta cell loss is typically less than previously thought in many patients. This highlights the importance of dysfunction of the residual beta cells as a factor in the development of hyperglycemia and diabetes symptoms at the time of diagnosis and possibly beyond[15]. A series of pilot experiments by nPOD investigators is testing the feasibility of generating pancreas slices to assess beta cell function from nPOD organ donors, without the confounding factors associated with islet isolation procedures. Islet function is also assessed in isolated islets when isolation can be performed.

Stage 5: CLINICAL ONSET OF T1D. Clinical onset of T1D, presenting characteristic signs and symptoms deriving from frank hyperglycemia, is thought to occur when approximately two-thirds of the islets have become insulin deficient[16]. Immediately after diagnosis, life-saving intervention with exogenous insulin therapy is started. Cases of death *at onset* are extremely rare nowadays, thanks to insulin treatment. nPOD has collected a large number of cases *post onset* of T1D, and a few have been recovered from the time of diagnosis or very close in time to it.

Stage 6: PERSISTENCE AND RECURRENCE OF AUTOIMMUNITY. T1D is a chronic disease. Minimal function and turnover of beta cells can be detected several decades after onset[17], and autoimmunity persists, as well. Perhaps T1D may be considered a chronic relapsing and remitting autoimmune disease, with flare-ups of autoimmune attacks counterbalanced (to an insufficient extent) by repair and regeneration processes. Pancreas transplantation or pancreatic islet transplantation are therapeutic options for patients who have developed kidney failure or have extreme difficulties in managing their diabetes. Recent studies show that in both pancreas and islet transplant recipients, autoimmunity can persist and at some point become reactivated, eventually leading to T1D recurrence. In pancreas transplant recipients, it is well documented that this may occur despite chronic treatment with immunosuppressive medication to prevent rejection[18]. nPOD has recovered pancreas transplant biopsy specimens from patients who have experienced T1D recurrence, and studies from nPOD investigators have shown pathological abnormalities that are largely overlapping to those found in spontaneous disease in the native pancreas. The study of transplanted human pancreatic tissues could provide insights also into mechanisms of beta cell regeneration and the pathogenesis of T1D. This is the focus of the **nPOD-Transplantation**[18] working group.

Big data, novel biomarkers of disease and therapeutic targets

The study of the appropriate samples with 'omics' methods and the use of unbiased analytical methods are providing novel insights for many human conditions, along with novel biomarkers and therapeutic targets for T1D. The 2016 nPOD meeting opened with the keynote lecture delivered by Dr. **Atul Butte** (University of California, San Francisco, USA). Dr. Butte has pioneered an approach for the discovery of molecular pathways associated with disease, of biomarkers and of therapeutic targets, that is based on the analysis of published omics data. He presented several examples of successful applications of this data mining strategy. For example, he presented expression-based genome-wide association studies (eGWAS) that enabled the identification of Vitamin D binding protein (VDBP) as a novel autoantigen in T1D[19]. Interestingly, VDBP is expressed in pancreatic islet α cells. Anti-VDBP autoantibodies could serve as an additional biomarker of T1D. Molecular pathways related to Vitamin D, which show a genetic association with T1D[20], are once again in the spotlight. Dr. **Linda Yip** (Garrison Fathman`s Lab, Stanford University, USA) reported the findings of gene expression studies in human pancreas and peripheral blood mononuclear cells (PBMCs) during the progression towards T1D. The group built on the success of previous expression studies that led to the implication of splicing variants of Adora1 and Deaf1 in disease progression[21]. Dr. Yip and colleagues used microarrays to study the gene expression profiles of pancreatic tissues (obtained from nPOD) and of peripheral blood mononuclear cells (PBMCs, obtained via the Type 1 Diabetes TrialNet https://www.diabetestrialnet.org). The analysis was performed in tissues from controls, nondiabetic autoantibody positive and T1D donors. The analysis provided a series of potential biomarkers of disease risk and progression. Overall, the gene expression profiles in pancreas and PBMCs have different patterns throughout the disease stages. Dr. **Ivan Gerling** (University of Tennessee, USA) analyzed gene expression profiles of islets obtained via laser capture microdissection. Islets were collected from donors with different stages of T1D: autoantibody positive cases without diabetes (pre-T1D), T1D cases at onset, T1D cases with long-standing disease, and pancreas transplant biopsies with recurrent T1D. The patterns of differentially expressed genes and pathways suggest that T1D pathogenesis could result from four interacting pathologies affecting beta cells: mitochondrial dysfunction and oxidative stress, viral infection, immune response, and replication/regeneration. Different combinations of these pathologies and pathways may contribute to disease heterogeneity. Gene expression datasets like those generated by Dr. Yip and by Dr. Gerling are useful for many investigators in the nPOD community, and they can be integrated with other datasets – such as those from genomic, epigenomic or proteomic studies. The integration of these 'omics' data will allow T1D investigators to formulate new questions and obtain deep insights from the available samples and databases. These are the focuses of the **nPOD-Omics** working group, a collaborative group focused on high throughput studies and dataset integration, which met and discussed strategies in a dedicated time slot during the meeting.

BOX 2. The George Eisenbarth Memorial Lecture

nPOD has instituted a lecture to honor the late Dr. George Eisenbarth, a true pioneer in T1D research who inspired and supported the creation of nPOD[4, 22]. Dr. **Gerald Nepom** (Benaroya Research Institute at Virginia Mason, USA) delivered the lecture and reviewed the use of immunological monitoring tools in the context of clinical trials. He highlighted the concept of combinatorial, sequential pharmacological regimens in which islet autoimmunity is initially arrested with a depleting or debulking agent, and a sustained therapeutic benefit is achieved in the long term by using immunoregulatory and/or anti-inflammatory agents. Alefacept, a promising drug that targets effector memory T lymphocytes, has shown efficacy in a recent clinical trial[23] and could be a good depleting agent with specificity for memory cells, which have been linked to T1D. Of note, findings presented at the nPOD meeting by Dr. Pugliese and his team demonstrate the presence of memory T cells in the insulitis lesion.

Immunology, autoimmunity and modified self-antigens (how and why beta cells become immunogenic?)

The concept that T1D is a chronic autoimmune disease was crystallized by the work of Dr. George Eisenbarth[3,4,22]. Dr. Eisenbarth realized that prevention and a cure for autoimmune T1D could be possible but would require a clear understanding of key pathogenic mechanisms of autoimmunity. The **nPOD-Autoimmunity** group studies autoreactive T cells, B cells and other types of immune cells. T cell receptor sequences, antigen specificities and functional properties of immune cells are at the center of interest for this working group. At the nPOD meeting there were also presentations from experts in other autoimmune diseases, describing groundbreaking findings and new concepts in the field of autoimmunity. Different autoimmune conditions share a similar genetic basis, immunological mechanisms, and potentially etiology – as suggested by simultaneous multiple autoimmune syndromic cases. A recurring theme emerged from several studies: autoimmunity may be triggered by *modified* forms of self-antigens. The factors that contribute to the generation of modified self-peptides could affect very different organs, but may ultimately trigger similar responses in immune cell populations.

Drs. John Harris (University of Massachusetts, USA) and **James Krueger** (Rockefeller University, USA) presented insights from other autoimmune diseases – respectively vitiligo, and psoriasis. Of note, Dr. Eisenbarth often paralleled the patchiness of the insulitis with the distribution of vitiligo. Dr. Harris highlighted how cellular stress, reactive oxygen species and abnormal activation of the unfolded protein response contribute to the development of vitiligo and autoimmune diseases in general[24]. He is studying strategies that block the Interferon-gamma/CXCL10 pathway that, in skin cells, is responsible for the recruitment of autoreactive CXCR3+ CD8+ T cells[25]. The same pathway (CXCL10/CXCR3) has also been linked to T1D, which has sparked interest in pharmacological strategies to block it[26]. Dr. Krueger was among the first to report that psoriasis is an autoimmune disease, which led to more effective therapies for this disease. Psoriasis is a chronic disease characterized by epidermal hyperplasia resulting from keratinocyte proliferation in response to cytokines released by T-cells and dendritic cells (DCs). Tofacitinib, a Janus kinase (JAK) inhibitor, attenuates JAK/STAT signaling in keratinocytes, reduces the numbers of pathologic T-cells and DCs and inhibits the IL-23/T_H17 pathway. Similar pathways may be involved in T1D, and the development of therapeutic approaches could benefit from cross-fertilization among the different fields of research in autoimmune diseases.

In autoimmune T1D, several outstanding questions remain in relation to how the immune system targets beta cells, and why immune cells exist that could target self-antigens in beta cells. **Dr. Emil Unanue** (Washington University, St Louis, USA) described a process through which the contents of the beta cell secretory granules become available to the immune system: beta cells transfer vesicles containing insulin and its catabolites to islet phagocytes for presentation to T cells[27]. Thus, beta cells appear to play an important role as source of autoantigens during the development of T1D. But why do we find immune cells targeting beta cell antigens? These cells have escaped thymic selection, or perhaps the antigens that these cells have

encountered during thymic selection differ from those encountered in peripheral tissues. There is a possibility that certain modified forms antigens could be generated specifically in beta cells, possibly only in certain conditions such as during an infection, stress, environmental factors or chemical reactions. The studies in rheumatoid arthritis presented by Dr. **Garrison Fathman** (Stanford University, USA) pointed in this direction. In this disease, modified antigens can develop in tissues as a result of chemical reactions. Citrullination, i.e. the conversion of the amino acid arginine to citrulline, is a post-translational modification causing protein structural changes due to increased hydrophobicity. Citrullination can occur during cell death or inflammation, and can alter antigen epitopes, rendering them immunogenic[28]. In rheumatoid arthritis, indeed, antibodies are raised against citrullinated forms of peptides. Anti-citrullinated protein antibodies are associated with more severe disease. Smoking and periodontal infection are two major environmental factors that contribute to the triggering of rheumatoid arthritis. Interestingly, citrullinated proteins appear in cellular debris after smoking. It is interesting to observe that antibodies to citrullinated proteins are associated with HLA 'shared epitope' alleles[28], strongly suggesting that a particular epitope modification could form complexes with certain forms of HLA, giving rise to a structure with unique structural and binding properties. Dr. **John Kappler** (Howard Hughes Medical Institute, National Jewish Health, Denver, USA) presented results related to modified forms of insulin antigens. Dr. Kappler studied the generation of super agonists for CD4+ T-cells from the proinsulin peptide in human and mouse models. Proinsulin fragments can undergo trans-peptidation, a protease-mediated peptide fusion, and can thus become highly immunogenic insulin-derived peptides. These fused peptides are potent stimulators of CD4 T cells, more so than the native peptides. Such post-translational modifications of peptides may occur only in the pancreas or in lymph nodes and not during thymic selection of T cells[29]. Novel studies by Dr. **Thomas Delong** and Dr. **Kathy Haskins** (University of Colorado, Denver, USA) support this notion. The group showed that beta cells could form highly immunogenic hybrid peptides via the fusion of fragments from two proteins. They identified autoantigenic epitopes that result from the combination of insulin and chromogranin A or insulin and neuropeptide Y peptides. These hybrid peptides acted as very potent activators of T-cell clones derived from non-obese diabetic (NOD) mice and human T1D patients[30]. We don't know yet whether transpeptidated peptides and hybrid fused peptides from beta cell proteins are the actual triggers of autoimmunity. Nonetheless, such peptides may form specifically in islets and not in the thymus; hence, T cells reactive to them would not be deleted during thymic selection. There is growing evidence that autoimmunity may be triggered by (and targeted to) modified forms of self-antigens. Chemical reactions, inflammation, cell death, viral infection or other environmental factors may facilitate the generation of such modified forms of self-peptides.

Dr. **Mia Smith** and Dr. **John Cambier** (University of Colorado-Denver, USA) reported a series of findings related to insulin autoimmunity and to the type of signals that could activate immune cells. Their studies analyzed insulin-binding B cells from healthy individuals, from individuals at risk of T1D (autoantibody positive), or those with clinically overt disease. Interestingly, B cells with high affinity receptors for insulin appear to be polyreactive, as they also bind to chromatin and lipopolysaccharide. In healthy individuals, insulin-binding B cells are anergic. In at-risk subjects and new-onset T1D patients there is a decrease in anergic insulin-binding B cells, supporting their activation; whereas at 1 year after diagnosis, the levels return to normal. The activation of these insulin-reactive B cells could derive from very different stimuli, including chromatin, which is commonly released after cell death in damaged or infected tissues, but also lipopolysaccharide, a molecule produced by gram-negative bacteria.

The role of pathogens and their interactions with innate immunity in the development of T1D may be central during the initial steps of disease development. Dr. **Decio Eizirik** (Universite Libre de Bruxelles, Belgium) highlighted the fact that more than 80% of the genes with variants associated with T1D risk are expressed in pancreatic islets, and that many of those genes are involved in innate immunity and antiviral responses[31]. The data presented support the hypothesis that genetic variants promote exaggerated innate beta cell reactivity to viral infections which could be a critical factor in the subsequent triggering of autoimmunity. On a similar note, Dr. **Oskar Skog** (Olle Korsgren's

group, Uppsala University, Sweden) observed the activation of innate antiviral pathways in pancreatic biopsies obtained from *living* patients with recent-onset of T1D (DiViD study). He reported that 23 out of 84 interferon-stimulated genes were found to be overexpressed in inflamed islets from T1D patients with recent onset. Viral sensor genes (IFIH1, MDA5, PKR and RIG-I) were also found to be overexpressed. Interferon gamma was overexpressed in islets and infiltrates from new-onset T1D cases. These findings indicate that innate antiviral pathways are active in T1D islet cells at the time of onset and warrant further investigation in search of viral entities. Such findings could have a positive impact in preventing or delaying T1D.

Dr. **Eoin McKinney** (University of Cambridge, U.K.) reported findings related to T cell exhaustion and autoimmunity. T cell exhaustion, a process that promotes viral persistence and is associated with a poor outcome in viral infections, is associated to better outcomes in autoimmunity. A low relapse rate was observed in multiple autoimmune conditions in the presence of a transcriptional signature indicating CD8+ T cell exhaustion, a signature inversely linked with a CD4+ T cell co-stimulation signature[32].

Dr. **Florence Anquetil** (Matthias Von Herrath lab, La Jolla Institute for Allergy and Immunology, USA) reported preliminary but intriguing findings on the immunoregulatory molecule Indoleamine 2,3-dioxygenase 1 (IDO1) and interleukin-6 (IL-6) – molecules that are expressed at different levels in islets of autoantibody positive cases without diabetes, T1D and T2D cases. IDO1 was found to be expressed in beta cells and was almost completely absent in pancreata from T1D patients. IL-6 appeared to be expressed at lower levels in islets from double antibody positive donors compared to controls, and at higher levels in T2D islets compared to controls. These findings suggest a relationship between these immune mediators, T1D and T2D.

Inflammation, innate immunity, and the gut

Inflammation and innate immunity seem to characterize early phases of the disease. In individuals at high risk for T1D and after T1D onset, a subset of islets containing beta cells appears to be inflamed. An important biomarker that seems to characterize prediabetic and diabetic beta cells, and that disappears after beta cells loss, is HLA overexpression. A fraction of pre-T1D beta cells overexpresses HLA class I and 'aberrantly' overexpress HLA class II before and during the insulitic invasion[33,34]. Dr. **Sarah Richardson** (University of Exeter, U.K.) presented results on the correlation of HLA class I hyperexpression and selective upregulation of Signal transducer and activator of transcription 1 (STAT1) in control and T1D donor beta cells. In islets from T1D patients, a beta-cell-specific hyperexpression of STAT1 was observed in parallel with HLA-class I hyperexpression, both at the gene and at the protein expression level. STAT1 activation is centrally involved in the signaling pathway activated by interferons, and it leads to an antiviral state and HLA-class I upregulation. STAT1 hyperexpression may thus be a driver of HLA-class I hyperexpression in T1D beta cells. This is in line with the report by Dr. **Oskar Skog**, who showed that other interferon-stimulated and viral sensor genes were overexpressed in inflamed islets from recent-onset T1D patients. Furthermore, he indicated that interferon gamma was overexpressed in islets and infiltrates from new-onset T1D cases, and this could contribute to the observed expression pattern. Dr. **Peter Butler** (University of California Los Angeles, USA) highlighted the need to remove the inflammation (the `fire`) before stimulating beta cell regeneration. Dr. Butler addressed the role of mitochondrial damage in T1D pathogenesis. Oxidative stress in beta cells induces mitochondrial fragmentation, activation of apoptosis and beta cell loss. Therefore, the more beta cells enter the cell cycle under stressed and inflamed conditions, the greater could be the loss of beta cells. The islet extracellular matrix represents a barrier to inflammatory cells and a substrate for the trafficking of immune cells. Dr. **Eva Korpos** (Lydia Sorokin`s lab, University of Muenster, Germany) showed that the peri-islet basement membrane and interstitial matrix components are lost at sites of leukocyte infiltration into the islet; this was observed both in nPOD donors with T1D and in pancreas transplant biopsies from patients with recurrent T1D[35].

Macrophages are among the first cell types to infiltrate the pancreatic islets during the disease process in NOD mice and BB rats. Dr. **Jason Gaglia** (Joslin Diabetes Center, USA) presented results related to noninvasive imaging of macrophages to study islet inflammation. Magnetic Resonance Imaging (MRI) of the clinically approved magnetic nanoparticle ferumoxytol enables imaging of mac-

rophages taking up nanoparticles in inflamed pancreatic lesions. Via this imaging method, he demonstrated increased accumulation of the nanoparticles in regions of the diseased pancreas compared to control tissue, as observed in mouse models of T1D and in a pilot human study in T1D patients with recent onset disease[36]. Other cell populations of the innate immunity seem to play a role in early phases of T1D. Dr. **Manuela Battaglia** (IRCCS San Raffaele Scientific Institute, Italy) reported a series of findings related to neutrophils. These cells are reduced in the peripheral blood of T1D patients at onset. Moreover, neutrophil counts seem to have an indirect correlation with the risk of developing T1D: the lower the neutrophil count, the higher the risk in autoantibody positive individuals[37]. Margination of neutrophils – i.e. the accumulation of neutrophils in the periphery of blood vessels due to adhesion to endothelial cells – can be observed in the pancreas of at risk subjects. Neutrophils accumulate in T1D nPOD pancreata, suggesting that low neutrophil counts in circulation are due to recruitment to the pancreas. Neutrophil Extracellular Traps are also observed in larger amounts in pancreata from first degree relative and T1D cases, compared to controls.

The pancreas may not be the only organ where inflammation occurs during T1D pathogenesis. Dr. **Shannon Wallet** (University of Florida, USA) highlighted that the gastrointestinal tract is chronically inflamed in T1D. Dr Wallet`s group has observed that intestinal epithelial cells have a central role in the innate immune dysfunction that characterizes the T1D intestine. Altered innate immune functions could lead to dysregulated adaptive immunity. Dr. **Christina Graves** (Shannon Wallet's group, University of Florida, USA) described the alterations that appear in T1D intestinal epithelial cells, including HLA class II overexpression and reduced goblet cell frequency. She reported an increased expression of TLR5 and TLR5-responsive inflammatory innate immune genes (Beta Defensin 2 and IL-17C). This was paralleled by an expansion of interferon-gamma producing intestinal immune populations. Intestinal epithelial cells could thus perceive the environment in an altered way in T1D, and they could initiate a miscommunication with intestinal immune cells. Moving forward, one of the key aims will be to determine whether such alterations in intestinal epithelial cells and intestinal innate immunity are a cause or a consequence of T1D.

Studies of isolated T1D islets

Dr. **Clayton Mathews** (University of Florida, USA) reported the key findings of an nPOD pilot project focused on live islets from T1D cadaveric donors. Pancreata from 3 young T1D donors (disease duration up to 7 years) were recovered and processed for islet isolation at the University of Pittsburgh and the Diabetes Research Institute-University of Miami. Islets were recovered and distributed for analysis to several laboratories, and a fraction of the islets had residual beta cells. Glucose-stimulated insulin release showed that T1D beta cells have a major loss of first-phase insulin release, which resembles observations in individuals at high risk of T1D and at clinical onset[14]. Moreover, islets displayed a striking bioenergetic failure, with defects in mitochondrial function and oxygen consumption rate. The islets showed a very high glycolytic rate. Hence, beta cells from T1D patients seem to have a major metabolic defect. Additional studies are ongoing, including gene expression studies in sorted live islet cells, and studies of T cell receptors from islet- infiltrating T cells. Studies of live pancreatic cells and tissues should provide critical information for the development of therapies and curative strategies for T1D.

Stem cells, progenitors and beta cell regeneration

A series of presentations focused on intriguing observations related to stem cells, progenitors and beta cell regeneration. Beta cell turnover can be detected several decades after T1D onset[17]. This suggests that repair and regeneration processes occur in the diseased pancreas, but they are insufficient to counterbalance the loss of beta cells resulting from autoimmunity. Autoimmunity and/or loss of beta cells could actually stimulate beta cell regeneration, as suggested by Dr. Ivan Gerling's findings in islets from autoantibody positive non-diabetic cases. The results reported by Dr. **Carol Lam** (Jake Kushner's Lab, *Baylor College of Medicine*, USA) challenge in part this idea. Dr. Lam showed that islet cell proliferation, measured with Ki67, was comparable in pancreata from T1D and nondiabetic adolescents. This finding suggests the absence of compensatory beta cell proliferation, at least after T1D onset. The same studies identified a highly proliferative population of α-like cells, expressing little to no glucagon and displaying cytoplasmic SOX9 positivity.

Dr. **Teresa Rodriguez-Calvo** (Matthias von Herrath`s group, La Jolla Institute for Allergy and Immunology, USA) showed that the insulin positive area is *increased* in pancreata from islet-autoantibody positive non-diabetic cases – cases that could represent the pre-diabetic phase in T1D – compared to controls. Interestingly, the insulin/proinsulin ratio was found to be inverted in autoantibody positive cases. Autoantibody positive cases thus seem to have more proinsulin than insulin, and larger areas positive for each molecule, compared to controls. This phenomenon was observed across the head, body and tail of the pancreas, but differences were more significant in the body and tail. These findings bring an important piece of information: compared to controls, autoantibody positive pancreata may not have lower beta cell mass, at least in early stages before the islet destructive process has advanced significantly. Whether this increased beta cell mass derives from hypertrophy or hyperplasia of beta cells in the pancreas will have to be determined. These findings also stimulate investigations aimed at analyzing the function of beta cells with inverted insulin/proinsulin ratio. An abundance of proinsulin compared to insulin (and C-peptide) can be considered a biomarker of beta cell endoplasmic reticulum dysfunction. A study on serum samples from the participants of the TrialNet Pathway to Prevention study provided results in line with those presented by Dr. Rodriguez-Calvo. Proinsulin and C-peptide were measured in the serum. The proinsulin-to-C-peptide ratio was found to be increased in antibody-positive subjects that progressed to diabetes compared with nonprogressors[38]. Thus, this ratio may have utility in predicting the onset of T1D in the presymptomatic phase.

Dr. **Alvin Powers** (Vanderbilt University, USA) emphasized inter-individual and age differences in islet cell mass. He showed that the beta cell mass varies by 3 to 5 times in adults, which may have implications for age of onset and rate of progression. This could ultimately result in different presentations of T1D. He also reported a series of changes that occur in islet cells throughout life. The studies suggest that islet cell composition is dynamic throughout life: relative proportions of endocrine cells change with age. Beta cells, in particular, increase progressively, whereas δ cells decrease. Interestingly, exendin 4 (a glucagon-like peptide-1 agonist) was found to promote proliferation of juvenile but not adult beta cells.

On the same topic, Dr. **Susan Bonner Weir** (Joslin Diabetes Center, Boston, USA) addressed key aspects of postnatal changes in human pancreas and islets. Postnatal development of the pancreas and islets is striking. Islet architecture changes with age, from simple rodent-like islets in young individuals to composite islets in adults. The beta cell ratio over total pancreas is maintained, but the pancreas grows from about 20 g at age 5 up to 120 g at age 26. Entirely new lobes and further branching are expected to form beyond age 5. Hence, completely new islets are expected to develop in postnatal life through a mechanism that is not based on beta cell replication[39]. In postnatal life, different progenitors could generate endocrine cells *de novo*, including pancreatic epithelial tip progenitors (as suggested by Dr. Powers), ductal cells[40], or other classes of progenitors[41,42]. The question is whether an activation of progenitors results in a compensatory *de novo* generation of beta cells in response to increased demand during the progression towards T1D and after onset. Mature endocrine cells may also act as facultative progenitors, and/or interconvert in different endocrine cells. Dr. **Pedro Herrera** (University of Geneva, Switzerland) showed that islet endocrine cells could interconvert, i.e. change cell identity, in response to injury. In mouse models engineered to have beta cells selectively ablated, newly formed beta cells frequently derived from preexisting alpha cells that spontaneously `reprogram` to beta cells. The form of insult determines the type of the response: if alloxan is used to cause beta cell loss, about 80% of alpha cells reprogram to beta cells, whereas if streptozotocin is used, alpha cells reprogram at low frequency. His group previously reported the interconversion of delta-to-beta cells in juvenile and alpha-to-beta cells in post-puberty rodent pancreata[43]. The appearance of bihormonal glucagon+/insulin+ cells in T2D pancreata and of somatostatin+/insulin+ cells in T1D pancreata suggests that interconversion occurs spontaneously also in diabetic patients.

Monogenic Diabetes

Approximately 1-5% of all cases of diabetes result from single-gene mutations and are thus monogenic forms of diabetes mellitus. Congenital mutations in one of a set of genes controlling beta cell function result in beta cell dysfunction[44], other mutations affect insulin sensitivity. Specific treatments exist for certain forms of monogenic

diabetes, and differential diagnosis is important to exclude involvement of autoimmunity. These monogenic forms of diabetes can be identified with genetic screening.

Dr. **May Sanyoura** (Siri Greeley`s group, University of Chicago, USA) reported that 4 cases with monogenic diabetes were identified in the nPOD tissue repository via genetic typing. All of these were autoantibody negative and had unique histological characteristics. Thus, new data are emerging about the pathology of monogenic forms of diabetes through the study of nPOD specimens.

Dr. **Lina Sui** (Dieter Egli`s lab, Columbia University, USA) presented the development of models of beta cell dysfunction via generation of induced Pluripotent Stem Cells (iPSC) from patients with monogenic diabetes. iPSC-derived beta cells reflect beta cell–autonomous phenotypes: cells from patients with SUR1 loss of function showed decreased insulin processing and increased proinsulin secretion. This approach enabled the creation of a platform for the analysis of the effect of specific genotypes on beta cell function. The team is centrally involved in the development of the Helmsley Cellular Research Hub (http://www.cellhub.org) that is generating iPSC lines from patients with T1D, and making them available to the scientific community.

Heterogeneity of T1D

There are reasons to believe that what we call `T1D` today is actually composed of a range of different diseases. Compared to monogenic diabetes, autoimmune T1D is of multifactorial etiology, and genetic studies indicate that variants in a large number of genes confer increased risk. The combination of the genetic variants in a specific individual will determine not only the risk to develop T1D, but also the pace of progression and the form of the disease. Recognition of this heterogeneity has implications for diagnosis and treatment. As noted above, Dr. **Ivan Gerling** (University of Tennessee, USA), presented results that four interacting pathologies may affect beta cells in T1D and different combinations of these pathways may contribute to the disease heterogeneity. The issue of heterogeneity was further discussed by Dr. **Desmond Schatz** (University of Florida, USA), who highlighted the major impact of this on disease treatment. There is a spectrum of disease presentations throughout ages. When T1D onset occurs very early in childhood, beta cell loss seems to progress very rapidly. At the opposite side of the spectrum, T1D can occur in adults (Latent or late-onset autoimmune diabetes of adults, LADA), but it often shows a slower course of onset and it has a milder presentation. Moreover, a historic and sharp rise in obesity occurred among children and adults over recent decades, and this complicates differential diagnosis of T1D/T2D, especially in patients over 30 years of age (a note highlighted also by Dr. **Richard Oram**). The observed spectrum of presentations may derive from different disease processes. In support of the concept of different disease processes, Dr. Schatz reported that the incidence of insulin autoantibodies (IAA) as a first autoantibody declines with age, whereas that of Glutamic acid decarboxylase antibodies (GADA) increases with age. Moreover, anti-CD20 (Rituximab) treatment showed a positive effect only when administered to children. Dr. **Noel Morgan** (University of Exeter, UK) reported findings related to different insulitic profiles and to the association of different compositions of insulitic lesions with the level of beta-cell loss and age at T1D onset. The high CD20+/CD4+ ratio in young T1D patients was associated with a more aggressive disease phenotype, more rapid progression and more profound beta cell loss[45]. The high CD20+/CD4+ ratio in young cases could explain the responsivity to anti-CD20 (Rituximab) treatment observed in young patients. Dr. **Richard Oram** (University of Exeter, UK) presented findings resulting from the use of the Type 1 diabetes genetic risk score on a cohort of 150,000 adults. The findings indicate that T1D is distributed evenly within the first 6 decades of life, but after 30 years of age an increase in T2D causes mistakes in clinical diagnosis and treatment.

Inspiring a new generation of T1D researchers

The nPOD Meeting organizers actively promote the participation of junior investigators and this year planned a special session dedicated to them, which included a lecture followed by an interactive session. Dr. **Aldo Rossini** (Joslin Diabetes Center, USA), a pioneer in the field of animal models of T1D, gave a special lecture to the young investigators. He presented an exquisite overview of the milestones in T1D research and therapy, with special attention to open questions that still need satisfactory explanations and to the problems that young investigators are facing. Dr. Rossini and the audience commented on the issues and frustrations related to the current research funding system, which largely

fails in supporting the youngest, most dynamic and creative minds. Dr. Rossini highlighted how passion, curiosity and gut feelings could lead to breakthroughs in research and therapy.

Dr. **Alberto Pugliese** (University of Miami, USA) and Dr. **Mark Atkinson** (University of Florida, USA), co-executive directors of nPOD, concluded the meetings with remarks on the ongoing questions that require collaborative efforts. nPOD has enabled unprecedented studies on very rare tissues – those derived from organ donors with T1D and with risk for the disease. Investigators from around the world joined their forces and launched a team science effort aimed at understanding the etiopathogenesis of T1D. The 8th nPOD Annual Meeting revealed new insights on human *T1D,* covering multiple aspects of beta cell biology and islet autoimmunity. We believe that the data being generated and shared by nPOD investigators will be instrumental in translating findings into better therapies, possibly even a cure or prevention, for T1D.

Acknowledgements
The authors would like to thank Dr. Alberto Pugliese, Dr. Mark Atkinson and Dr. Amanda Posgai for critically reviewing the paper.

Conflict of Interests
The Authors declare that they have no conflict of interests.

References
1. Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 1986; 314: 1360-1368.
2. Skyler JS, Ricordi C. Stopping type 1 diabetes: attempts to prevent or cure type 1 diabetes in man. Diabetes 2011; 60: 1-8.
3. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2014; 383: 69-82.
4. Eisenbarth GS. Banting Lecture 2009: an unfinished journey: molecular pathogenesis to prevention of type 1A diabetes. Diabetes 2010; 59: 759-774.
5. Pugliese A, Yang M, Kusmarteva I, Heiple T, Vendrame F, Wasserfall C, Rowe P, Moraski JM, Ball S, Jebson L, Schatz DA, Gianani R, Burke GW, Nierras C, Staeva T, Kaddis JS, Campbell-Thompson M, Atkinson MA. The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD) Program: goals, operational model and emerging findings. Pediatr Diabetes 2014; 15: 1-9.
6. Campbell-Thompson M, Wasserfall C, Kaddis J, Albanese-O'Neill A, Staeva T, Nierras C, Moraski J, Rowe P, Gianani R, Eisenbarth G, Crawford J, Schatz D, Pugliese A, Atkinson M. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev 2012; 28: 608-617.
7. Kaddis JS, Pugliese A, Atkinson MA. A run on the biobank: what have we learned about type 1 diabetes from the nPOD tissue repository? Curr Opin Endocrinol Diabetes Obes 2015; 22: 290-295.
8. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009; 360: 1646-1654.
9. Todd JA. Etiology of type 1 diabetes. Immunity 2010; 32: 457-467.
10. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; 2: 1279-1283.
11. In't Veld P, Lievens D, De Grijse J, 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: 2400-2404.
12. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965; 14: 619-633.
13. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 2012; 209: 51-60.
14. Sosenko JM, Skyler JS, Beam CA, Krischer JP, Greenbaum CJ, Mahon J, Rafkin LE, Matheson D, Herold KC, Palmer JP; Type 1 Diabetes TrialNet and Diabetes Prevention Trial–Type 1 Study Groups. Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in diabetes prevention trial-type 1 participants. Diabetes 2013; 62: 4179-4183.
15. Pugliese A. Insulitis in the pathogenesis of type 1 diabetes. Pediatr Diabetes 2016; 17 Suppl 22: 31-36.
16. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia 1986; 29: 267-274.
17. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL. Residual insulin production and pancreatic ss-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 2010; 59: 2846-2853.
18. Pugliese A, Vendrame F, Reijonen H, Atkinson MA, Campbell-Thompson M, Burke GW. New insight on human type 1 diabetes biology: nPOD and nPOD-transplantation. Curr Diab Rep 2014; 14: 530.
19. Kodama K, Zhao Z, Toda K, Yip L, Fuhlbrigge R, Miao D, Fathman CG, Yamada S, Butte AJ, Yu L. Expression-based genome-wide association study links vitamin D-binding protein with autoantigenicity in Type 1 Diabetes. Diabetes 2016; 65: 1341-1349.
20. Cooper JD, Smyth DJ, Walker NM, Stevens H, Burren OS, Wallace C, Greissl C, Ramos-Lopez E, Hyppönen E, Dunger DB, Spector TD, Ouwehand WH, Wang TJ, Badenhoop K, Todd JA. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. Diabetes 2011; 60: 1624-1631.
21. Yip L, Fathman CG. Type 1 diabetes in mice and men: gene expression profiling to investigate disease pathogenesis. Immunol Res 2014; 58: 340-350.
22. Pugliese A, Skyler JS. George S. Eisenbarth: insulin and type 1 diabetes. Diabetes Care 2013; 36: 1437-1442.

23. Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, Griffin KJ, Tsalikian E, Gottlieb PA, Greenbaum CJ, Sherry NA, Moore WV, Monzavi R, Willi SM, Raskin P, Keyes-Elstein L, Long SA, Kanaparthi S, Lim N, Phippard D, Soppe CL, Fitzgibbon ML, McNamara J, Nepom GT, Ehlers MR. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. J Clin Invest 2015; 125: 3285-3296.
24. Harris JE. Cellular stress and innate inflammation in organ-specific autoimmunity: lessons learned from vitiligo. Immunol Rev 2016; 269: 11-25.
25. Rashighi M, Harris JE. Interfering with the IFN-gamma/CXCL10 pathway to develop new targeted treatments for vitiligo. Ann Transl Med 2015; 3: 343.
26. Antonelli A, Ferrari SM, Corrado A, Ferrannini E, Fallahi P. CXCR3, CXCL10 and type 1 diabetes. Cytokine Growth Factor Rev 2014; 25: 57-65.
27. Vomund AN, Zinselmeyer BH, Hughes J, Calderon B, Valderrama C, Ferris ST, Wan X, Kanekura K, Carrero JA, Urano F, Unanue ER. Beta cells transfer vesicles containing insulin to phagocytes for presentation to T cells. Proc Natl Acad Sci U S A 2015; 112: E5496-5502.
28. Wegner N, Lundberg K, Kinloch A, Fisher B, Malmström V, Feldmann M, Venables PJ. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev 2010; 233: 34-54.
29. Jin N, Wang Y, Crawford F, White J, Marrack P, Dai S, Kappler JW N-terminal additions to the WE14 peptide of chromogranin A create strong autoantigen agonists in type 1 diabetes. Proc Natl Acad Sci U S A 2015; 112: 13318-13323.
30. Delong T, Wiles TA, Baker RL, Bradley B, Barbour G, Reisdorph R, Armstrong M, Powell RL, Reisdorph N, Kumar N, Elso CM, DeNicola M, Bottino R, Powers AC, Harlan DM, Kent SC, Mannering SI, Haskins K. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. Science 2016; 351: 711-714.
31. Santin I, Dos Santos RS, Eizirik DL. Pancreatic beta cell survival and signaling pathways: effects of Type 1 Diabetes-associated genetic variants. Methods Mol Biol 2016; 1433: 21-54.
32. McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. Nature 2015; 523: 612-616.
33. Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 1985; 313: 353-360.
34. Foulis AK, Farquharson MA. Aberrant expression of HLA-DR antigens by insulin-containing beta-cells in recent-onset type I diabetes mellitus. Diabetes 1986; 35: 1215-1224.
35. Korpos É, 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: 531-542.
36. Gaglia JL, Harisinghani M, Aganj I, Wojtkiewicz GR, Hedgire S, Benoist C, Mathis D, Weissleder R. Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. Proc Natl Acad Sci U S A 2015; 112: 2139-2144.
37. Valle A, Giamporcaro GM, Scavini M, Stabilini A, Grogan P, Bianconi E, Sebastiani G, Masini M, Maugeri N, Porretti L, Bonfanti R, Meschi F, De Pellegrin M, Lesma A, Rossini S, Piemonti L, Marchetti P, Dotta F, Bosi E, Battaglia M. Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. Diabetes 2013; 62: 2072-2077.
38. Sims EK, Chaudhry Z, Watkins R, Syed F, Blum J, Ouyang F, Perkins SM, Mirmira RG, Sosenko J, DiMeglio LA, Evans-Molina C. Elevations in the fasting serum proinsulin-to-C-peptide ratio precede the onset of Type 1 Diabetes. Diabetes Care 2016; 39: 1519-1526.
39. Bonner-Weir S, Aguayo-Mazzucato C, Weir GC. Dynamic development of the pancreas from birth to adulthood. Ups J Med Sci 2016; 121: 155-158.
40. Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T, Sharma A. The pancreatic ductal epithelium serves as a potential pool of progenitor cells. Pediatr Diabetes 2004; 5 Suppl 2: 16-22.
41. Wang Y, Lanzoni G, Carpino G, Cui CB, Dominguez-Bendala J, Wauthier E, Cardinale V, Oikawa T, Pileggi A, Gerber D, Furth ME, Alvaro D, Gaudio E, Inverardi L, Reid LM. Biliary tree stem cells, precursors to pancreatic committed progenitors: evidence for possible life-long pancreatic organogenesis. Stem Cells 2013; 31: 1966-1979.
42. Klein D, Álvarez-Cubela S, Lanzoni G, Vargas N, Prabakar KR, Boulina M, Ricordi C, Inverardi L, Pastori RL, Domínguez-Bendala J. BMP-7 induces adult human pancreatic exocrine-to-endocrine conversion. Diabetes 2015; 64: 4123-4134.
43. Chera S, Baronnier D, Ghila L, Cigliola V, Jensen JN, Gu G, Furuyama K, Thorel F, Gribble FM, Reimann F, Herrera PL. Diabetes recovery by age-dependent conversion of pancreatic delta-cells into insulin producers. Nature 2014; 514: 503-507.
44. Yang Y, Chan L. Monogenic Diabetes: What It Teaches Us on the Common Forms of Type 1 and Type 2 Diabetes. Endocr Rev 2016; 37: 190-222.
45. Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, Morgan NG. Differential insulitic profiles determine the extent of beta-cell destruction and the age at onset of Type 1 Diabetes. Diabetes 2016; 65: 1362-1369.