Diabetes mellitus. 2014;(2):20–28

Современные возможности применения стволовых клеток при сахарном диабете

[1]Дедов И.И., [2]Лисуков И.А., [1]Лаптев Д.Н.

[1]ФГБУ Эндокринологический научный центр, Москва
(директор – академик РАН и РАМН И.И. Дедов)
[2]Центр онкогематологии и трансплантологии СЗГМУ им. И.И. Мечникова, Санкт-Петербург
(ректор – профессор д.м.н. О.Г. Хурцилава)

Сахарный диабет (СД) характеризуется относительным или абсолютным дефицитом инсулина. Современные методы лечения СД не позволяют добиться нормального уровня глюкозы в крови без эпизодов гипо- и гипергликемии и полностью предотвратить развитие осложнений СД. Замещение β-клеток (пересадка поджелудочной железы или β-клеток) сопровождается осложнениями, требует пожизненной иммуносупрессивной терапии, при этом далеко не всегда достигается инсулинонезависимость, а также имеется существенный дефицит доноров. Выходом из сложившейся ситуации может стать применение стволовых клеток (СК), которые лишены этих недостатков. Аллогенные трансплантации СК осложнены иммунным отторжением трансплантата, а использование эмбриональных СК – этическими аспектами, сложностью отбора клеточных линий и риском возникновения тератом. Данный обзор посвящен возможностям использования тканевых СК для аутологичной трансплантации с целью восстановления пула β-клеток, а также иммунологической реконституции, и для модуляции аутоиммунного процесса при СД.
Ключевые слова: *сахарный диабет; β-клетки; стволовые клетки; пуповинная кровь; кроветворные клетки*

Modern possibilities for using stem cells in diabetes mellitus

Dedov I.I.[1], Lisukov I.A. [2], Laptev D.N. [1]
[1]Endocrinology Research Centre, Moscow, Russian Federation
[2]Centre for Oncogematology and Transplantology, Northwestern State Medical University named after I.I. Mechnikov, St. Petersburg

Diabetes mellitus (DM) is characterised by relative or absolute insulin deficiency. The currently available treatment methods for DM cannot provide normal blood glucose level without hypo- or hyperglycaemia episodes, thus failing to completely prevent the development of diabetic complications. Replacement of β cells (transplantation of the pancreas or β cells) is accompanied by complications and requires life-long immunosuppressive therapy that is not always followed by restoration of insulin independence; there is also a substantial deficit of donors. Stem cells do not cause such negative effects and can be used in therapy to avoid such problems. Allogeneic stem cell transplantation is complicated by immune rejection of a transplant, whereas the use of embryonic stem cells is associated with ethical concerns, complicated cell line selection, and risk of teratoma formation. The present review focuses on therapeutic pathways of autologous transplantation of tissue stem cells in order to restore the β-cell pool, for immune reconstitution and modulation of the immune response in DM patients.
Keywords: *diabetes mellitus; β-cells; stem cells; umbilical cord blood; haematopoietic cells*

DOI: 10.14341/DM2014220-28

Diabetes mellitus. 2014;(2):20–28

Diabetes mellitus (DM) has an exceptionally complex pathogenesis, including genetic and epigenetic disorders that lead to the key pathogenic stage of the disease, hyperglycaemia (glucose toxicity). In type 1 DM (T1DM), β-cells are progressively destroyed through autoimmune processes. On the other hand, the main cause of type 2 DM (T2DM) is insulin resistance of peripheral tissues. To overcome the high tolerance threshold to glucose in tissues, β-cells function in a 'fast-paced' manner, which eventually leads to their depletion insulin deficiency. Since the discovery of insulin in 1920, insulin replacement therapy remains the main treatment option for DM. However, even intensified insulin therapy does not achieve ideal accuracy and circadian periodicity of insulin levels typical for healthy people, thus leading to inevitable episodes of hypo- and hyperglycaemia and development of vascular complications [1].

During the past years, intensive research has been conducted to identify a radical treatment for T1DM, such as using cell-based technologies and methods for growing pancreas from embryonic stem cells (SCs). Cell therapy based on the replacement of β-cells could become the ideal approach for treating patients with T1DM. Complete transplantation of the pancreas, which was first performed in 1966, is one of the β-cell replacement strategies used at present [2]. As of 2008, more than 30,000 pancreatic transplantation had been performed worldwide [3, 4]. Recent evidence demonstrates that patients with normal HbA_{1c} levels who underwent pancreatic transplantation were completely insulin-independent for more than 2 years after the procedure [5]. However, pancreatic transplantation requires major surgery characterised by a mortality rate of 1%–3%, severe cardiovascular complications and lifelong immunosuppressive therapy (IST). Therefore, pancreatic transplantation is mainly recommended for patients with T1DM with severe complications (terminal stage of diabetic nephropathy, 'brittle' diabetes with, frequent episodes of severe hypoglycaemia and disrupted perception of hypoglycaemia) [6]. Attempts have been made to overcome such complications by transplanting pancreatic islets. However, these technologies, despite the successful increase in C-peptide levels in blood, failed to provide insulin independence (until 1989) and required high doses of immunosuppressive drugs [7]. In 2000, the Edmonton protocol was developed, introducing corticosteroid-free immunosuppression. Although the Edmonton protocol induced insulin-independence in 7 patients for 1 year (11.9 months), this result was difficult to reproduce, and the 9-year transplant survival rate was <10% [8]. Thus, replacement of β-cells is accompanied by complications, requires lifelong IST and insulin independence is not always achieved. Moreover, this treatment faces problems such as a lack of donors for transplantation and sustained autoimmunity in patients. Use of SCs may serve as a solution to this problem.

SCs offer several advantages compared with β-cell transplantation as follows:

1) abundantly available SC donors,
2) a permanent source of β-cells,
3) minimal dependence (up to complete independence) on immunosuppressive drugs,
4) suppression of autoimmunity and reconstitution of the immune system after IST [9].

SCs are derived from embryonic (undifferentiated pluripotent embryonic SCs derived from blastocysts) and adult tissues (more highly differentiated SCs derived from various sources such as blood, bone marrow, liver, PG).

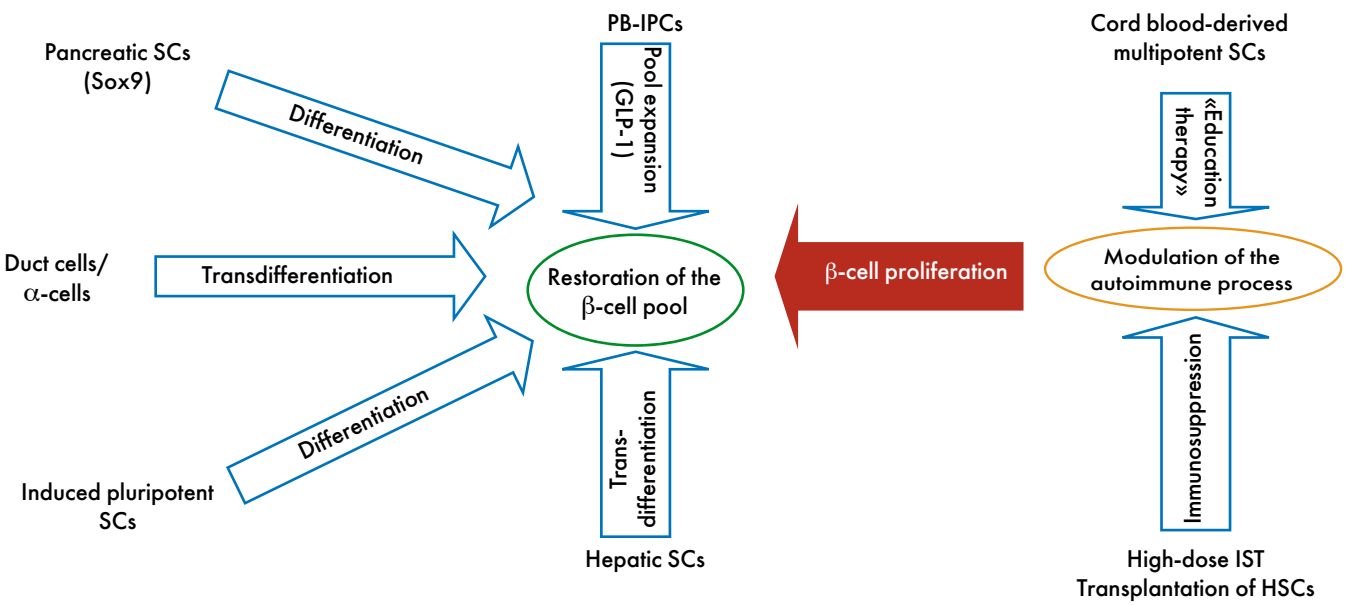

Figure 1. Approaches to restore the β-cell pool using stem cell technology. Explanations are provided in the text.
Abbreviations: SCs: stem cells; PB-IPCs: peripheral blood-derived insulin-producing cells; IST: immunosuppressive therapy; HSCs: haematopoietic stem cells.

DOI: 10.14341/DM2014220-28

SC transplantation is of 2 main types: autologous (when a recipient is simultaneously a donor of SCs) and allogeneic (when another person is the donor). The main problem with allogeneic transplantation is the immune rejection of a transplant, which occurs even when embryonic SCs are used. Thus, it is important to develop methods for protecting a transplant against immune rejection by the recipient, or a patient must undergo lifelong IST. The use of embryonic SCs is associated with a number of serious problems as follows:

1) Undifferentiated embryonic SCs may form teratomas in vivo.
2) There are ethical concerns regarding the methods used for obtaining embryonic SCs.
3) The ability of various SCs to differentiate into β-cells varies; therefore, a large number of embryonic SC lines must be studied to provide the appropriate differentiated phenotype.

The use of autologous SCs appears more promising for obtaining insulin-producing cells and to reconstruct the immune system. The current review focuses on the possibilities of autologous transplantation of adult tissue SCs for restoring the β-cell pool, immunologic reconstitution and modulation of autoimmunity in patients with DM (Fig. 1).

STEM CELLS

Under certain physiological conditions such as obesity and pregnancy, the β-cell pool can expand within physiological limits [10]. Further, significant regeneration of the pancreatic tissue, including β-cells, occurs in rats after 90% pancreatectomy [10]. According to the results of studies based on tracing genetic lineage, existing β-cells are the major source of new β-cells in vivo. Moreover, SCs participate in β-cell regeneration [11, 12].

Pancreatic ductal cells are capable of differentiating into β-cells. Pancreatic duct ligation in rats leads to substantial hyperplasia, mainly because β-like cells which secrete insulin in response to increased glycaemia but do not express all β-cell-specific markers. Therefore, hyperplasia cannot be explained only by the proliferation of existing β-cells and is possibly caused by SCs [13]. Regeneration may occur because of transdifferentiation and proliferation of pancreas exocrine duct cells. Duct cells isolated ex vivo from the exocrine pancreatic tissue of healthy donors re-express the transcription factor PDX1, the key component responsible for the development of pancreas, which is indicative of their potential to serve as SCs [14]. However, recent data cast doubt on the possibility of using duct cells as a source of β-cells.

Progenitor SCs expressing the transcription factor SOX9 may serve as a physiological source of β-cells. However, several ex vivo experiments on stimulating regeneration (e.g. pancreatic duct ligation, partial pancreatectomy) do not prove that SOX9-positive ductal and acinar cells are a source of β-cells [15]. Moreover, it was demonstrated that even after stimulation of regeneration, ductal and acinar cells of an adult mouse did not express the transcription factor HNF1β, which is expressed only by pancreatic duct cells [16]. Several research groups have isolated multipotent SCs directly from pancreatic islets. Some cells re-express the transcription factor NEUROG3 after pancreatic duct ligation. These multipotent cells are capable of differentiation into any of the 4 types of endocrine pancreatic cells (α-, β-, δ- and PP) [17].

Despite the presence of potential candidates in pancreas as new sources of β-cells, the source of these cells remains uncertain. A previous study has found that β-cell growth and differentiation in the pancreatic islets of adult mice is maintained only by mature β-cells [18]. Further studies should determine whether patients with DM have pancreatic SCs as well as the mechanisms that stimulate their differentiation in vivo. Another possible direction is to develop protocols for isolating and differentiating the cells in vitro for transplantation.

INDUCED PLURIPOTENT STEM CELLS

Pluripotent cells induced by transcription factors represent a potential source of β-cells. In 2006, Yamanaka et al. demonstrated the possibility of reprogramming human and mouse somatic cells to induced pluripotent SCs (iPSCs) using enforced expression of 4 genes *(OCT4, SOX2, KLF4* and *c-MYC)* [19, 20]. These cells express SC-specific markers and exhibit the morphology of embryonic SCs. iPSCs were subsequently obtained by reprogramming other types of somatic cells [21]. iPSCs can generate SCs for further differentiation into β-cells [22]. Further, mouse skin fibroblasts reprogrammed by transducing them with retroviral vectors expressing transcription factors can be used for further selective differentiation of SCs into β-like cells. The resulting cells are capable of secreting insulin, depending on the level of glycaemia, and if transplanted into animals with T2DM, decreasing HbA_{1c} levels and correcting hyperglycaemia [23]. Further, insulin-producing cells are generated from reprogrammed fibroblasts isolated from patients with T1DM [24], and human β-cells can be successfully reprogrammed into iPSCs. Programmed iPSCs acquire markers of pluripotent cells with the potential to differentiate into the 3 embryonic germ layers. These cells exhibit greater potential for differentiation into insulin-producing cells in vitro and in vivo compared with iPSCs from the same patient that are not derived from β-cells [25]. These findings demonstrate the existence of epigenetic memory of β-cell-derived iPSCs that is responsible for their differentiation into insulin-producing cells.

However, a significant disadvantage of this method of obtaining iPSCs is the use of viruses (retroviruses and lentiviruses) that irreversibly integrate their genetic material (including oncogenes) into the genome, thus introducing a risk of neoplasia after reactivation of viral transgenes. It is safer to use adenoviral and episomal vectors that activate transient expression of exogenous genes without integrating into the host genome [26]. Another relatively safe method to induce a pluripotent SC is to reprogramme

DOI: 10.14341/DM2014220-28

a spermatogonial cell into an embryonic-like SC without genetic transformation. Thus, embryonic SCs differentiate into pluripotent SCs in the presence of appropriate growth factors and may further differentiate into any of the 3 embryonic germ layers [27]. Further, various chemicals that do not alter genome structure can be used for reprogramming [28].

HEPATIC STEM CELLS

Because the origin of PG is endodermal, the liver is a potential source of pancreatic SCs. Integration mediated by the gene encoding transcription factor PDX1 in mice leads to the transdifferentiation of hepatocytes into progenitor β-cells that express numerous pancreatic genes [29]. Moreover, PDX1 induces its own expression (autoinduction), which presumably contributes to prolonged survival of transdifferentiated cells. These cells secrete insulin in amounts sufficient to prevent streptozotocin-induced (streptozotocin is toxic to β-cells and causes T1DM in mice) hyperglycaemia. High glucose levels in vitro or hyperglycaemia in vivo are required for further differentiation and maturation of progenitor β-cells [30]. However, it is unclear whether it is possible to culture modified liver cells in vitro to produce a cell mass sufficient for transplantation [31].

UMBILICAL CORD STEM CELLS

By the end of 2009, approximately 20,000 transplants of umbilical cord blood [32] containing haematopoietic stem cells (HSCs) and mesenchymal SCs were performed worldwide. Umbilical cord blood transplantation is used to treat hematopoietic cancers, immunodeficiency, haemoglobinopathy and genetic diseases. Umbilical cord blood transplants are used to treat paediatric and adult patients with DM. The safety of autologous donation of umbilical cord blood SCs for children older than 1 year (mean age, 5.5 years) has been confirmed soon after the manifestation of T1DM [33]. However, the clinical outcomes of this study were unsatisfactory. For example, 12 months after administration of umbilical cord blood SCs, there was no improvement in C-peptide secretion, no decrease in exogenous levels of insulin and HbA_{1c} and no increase in the levels of regulatory T-cells. Moreover, an increase in the levels of regulatory T-cells was noted during the months 6 and 9 of follow-up. These disappointing results may be explained by an insufficient amount of transplanted cells, indicated by the increase in the levels of regulatory T-cells during months 6 and 9 and a decrease in the levels 12 months after treatment.

Recently, Zhao et al [9] describes an alternative method of using cord blood derived stem cells. The first step of the procedure [9] includes isolation of a specific population known as 'cord blood-derived multipotent SCs' from umbilical cord blood SCs. These cells express a series of embryonic cell markers (OCT4, NANOG, SSEA3 and SSEA4) on their surface as well as leukocyte common antigen CD45 but not haematopoietic markers. These cells comprise 0.1% of the total amount of SCs. SCs are transferred into a so-called Stem Cell Educator device. The transplantation procedure separates lymphocytes from the recipient's peripheral blood followed by slow passage of the cells through the Stem Cell Educator device where they contact SCs and are returned to the recipient's bloodstream. SCs remain in the device and are not introduced into the bloodstream. The procedure is performed using a closed-loop system for 8–9 h.

Such a technique was utilised to treat adult patients with T1DM [34] and T2DM (results were presented on scientific session 'Umbilical cord blood stem cells and cellular technologies. Regenerative therapy' , St.Petersburg, Russia, June 2013) in the first and second phases of a clinical trial. Single treatment using the Stem Cell Educator led to the reversal of autoimmunity and partial regeneration of β-cells, which improved glycaemic control and reduced the daily dose of insulin. In patients with T1DM with no residual C-peptide secretion (fasting C-peptide levels of <0.01 ng/ml), daily insulin and HbA_{1c} doses were reduced by an average of 25% and 1.9%, respectively. In contrast, patients with residual C-peptide secretion (average dose of C-peptide = 0.33 ng/ml), the average doses of insulin and HbA_{1c} were reduced by 38% and 1.6%, respectively. Patients with T1DM expressed increased levels of co-stimulatory molecules (CD28 and ICOS) in lymphocytes, increased numbers of regulatory T-cells (CD4+CD25+Foxp3+) and restored Th1/Th2/Th3 cytokine balance after treatment, indicating reversal of autoimmunity. HbA_{1c} levels were reduced in patients with T2DM who underwent treatment, smaller daily insulin doses were required and chronic inflammation was reduced as indicated by the expression of CTLA-4, CD80/CD86, CD28 and TGF-β1 secretion. No statistical difference among these indicators was identified in the placebo group. Fasting and stimulated C-peptide secretion in the T1DM group with no residual C-peptide secretion may indicate the existence of regeneration mechanisms triggered by the modulation of the autoimmune process. The regenerated cells may be derived from multiple endogenous resources, as described above, and peripheral blood-derived insulin-producing cells..

PERIPHERAL BLOOD STEM CELLS

Peripheral blood-derived insulin-producing cells (PB-IPC) first isolated in 2006, are promising candidates as a source of autologous insulin-producing SCs [35, 36]. PB-IPCs exhibit properties of embryonic and HSCs, along with a high potential for producing insulin. These cells express insulin mRNA and the transcription factors MAFA and NKX6-1 [35] as well as somatostatin (produced by δ-cells) and ghrelin (produced by ε-cells). However, they do not express glucagon (produced by α-cells), pancreatic polypeptide (produced by PP-cells) and the transcription

DOI: 10.14341/DM2014220-28

factors PDX1, NEUROD1, NKX2-2 that are endocrine cell-specific. These cells proliferate in culture to significant numbers and produce insulin [35]. Moreover, electron microscopy shows numerous granules with a diameter of 200–300 nm in the cytoplasm of PB-IPCs, with some granules having a halo structure, which is unique to human β-cells. The presence of insulin granules is supported by observations using transmission electron microscopy. PB-IPCs express other β-cell specific markers, including glucose transporter GLUT2, sulphonylurea receptor SUR1 (K^+ATF channel protein responsible for insulin secretion) and glucokinase regulatory protein (GCKR).

PB-IPCs express characteristic phenotypic markers of HSCs, such as tetraspanin CD9, leukocyte common antigen CD45 and SC growth factor receptor CD117. Further, PB-IPCs do not express the HSC marker CD34 and lymphocyte markers CD3 (T-cells) and CD20 (B-cells). CD45 expression indicates that PB-IPCs represent haematopoietic ($CD45^+$) cells rather than mesenchymal ($CD45^-$) cells that circulate in peripheral blood. Further, these cells express embryonic SC-specific transcription factors OCT4 and NANOG. This phenotype is exhibited by umbilical cord blood SCs [36].

In a previous study, transplantation of the PB-IPCs population in NOD mice decreased glycaemia by 20%–30% compared with control. Furthermore, the experiments showed a significant increase in human C-peptide levels compared to control group where C-peptide levels remained undetectable. The level of C-peptide after transplantation of approximately 5,000,000 PB-IPC corresponds to that of C-peptide after transplantation of 2,000 human islets [37]. One month after transplantation, human C-peptide-positive cells were detected in the pancreatic tissue, including residual pancreatic islets, which reside in close vicinity to glucagon-positive cells. Further, human C-peptide-positive cells were undetectable in other tissues. These findings indicate that PB-IPCs migrate to pancreatic islets after transplantation into the peritoneum. Therefore, migration (homing) of PB-IPCs into pancreatic islets is likely not random and is controlled by specific chemokines, which is confirmed by the presence of the chemokine SDF-1-specific receptor CXCR4 on the surface of PB-IPCs. In turn, SDF-1 plays a key role in the homing of HSCs [38]. Human and NOD mouse SDF-1 chemokines differ by a single amino acid; they are highly expressed in pancreatic tissues of NOD mice, confirming this mechanism. To develop the optimal transplantation protocols, it is necessary to identify mechanisms for increasing the pool of PB-IPCs in vitro as well as to conduct a series of studies on efficacy and safety.

AUTOLOGOUS TRANSPLANTATION OF HAEMATOPOIETIC STEM CELLS

In 1997, the European Group for Blood and Marrow Transplantation and the European League against Rheumatism (EBMT/EULAR Autoimmune Disease Stem Cell Project) presented a paper on the main principles of the efficacy and safety assessment of high-dose IST and HSC transplantation in autoimmune diseases (AUD) [39]. The key aspects of this programme included application of autologous SCs, standard protocols on pretransplantation preparation (conditioning protocols), peripheral HSC mobilisation techniques and protocols for separating a transplant from immunocompetent cells among others. By 2014, the International Bone Marrow Transplant Registry (IBMTR) included more than 2,000 patients with various AUDs (multiple sclerosis, systemic lupus erythematosus, systemic scleroderma and rheumatoid arthritis) and patients with T1DM.

HSCs derived from a patient's peripheral blood (PHSCs), isolated from bone marrow and mobilised into bloodstream using high doses of cyclophosphamide (2–4 g/m^2) and granulocyte colony-stimulating factor (G-CSF) or by G-CSF only, are used in most studies as a source of HSCs for autologous transplantation. The target number of PHSCs required for transplantation is >2–3 million $CD34^+$ HSCs per kg of recipient's weight. Mobilised PHSCs are collected from peripheral blood using leukapheresis with programmed blood cell separators, cryopreserved and subsequently used for pretransplantational high-dose IST.

Theoretically, intensification of IST up to complete immunoablation may eradicate autoreactive lymphocyte clones. Autologous transplantation with HSCs allows administration of such treatment in a relatively safe manner, and the duration of critical neutropenia is usually no longer than 10–14 days. In contrast, during immune reconstitution in the absence of inducers, reversion to latent AUDs with the restoration of immunological tolerance to autoantigens may occur. This effect is known as 'immune system reprogramming', because this process is considered most promising for achieving long-term remission or even cure of AUD. The protocols for pretransplantational high-dose IST in AUDs are empirically selected on the basis of HSC transplantation experience with haemoblastosis and aplastic anaemia. Most often, these protocols include high doses of alkylating cytostatics (cyclophosphamide). Several medical centres in the United States frequently use cyclophosphamide combined with total body irradiation (TBI) at 200–800 kGy [40].

Immunoablation followed by reprogramming of the immune system can be efficient in patients with T1DM if the pool of β-cells for further regeneration is relatively conserved. For example, studies conducted at the São Paulo University Medical School (Brazil) on the role of autologous transplantation of HSCs in early-stage T1DM are particularly interesting. The research protocol included mobilisation of PHSCs with 2 g/m^2 cyclophosphamide and G-CSF (filgrastim) at 10 μg/kg daily, separation of PHSCs and further cryoconservation. Two weeks after completing cell collection, cyclophosphamide at 200 mg/kg and rabbit antithymocyte globulin (ATG) at 4.5 mg/kg were used as a pretransplantation conditioning regimen. After conditioning, 4.9–23.1 × 10^6 $CD34^+$ cells/kg of thawed PHSCs were reinfused intravenously. Patients were

DOI: 10.14341/DM2014220-28

hospitalised for 15–24 days, while neutrophil recovery (≥0.5 × 10^9/L) required 8–11 days.

A similar procedure was used to treat 23 anti-GAD+ patients aged 13–31 years with recently diagnosed (≤6 weeks) T1DM with no ketoacidosis signs. Average blood glucose and HbA_{1c} levels were 395.6 mg/dL and 8.4%, respectively. During treatment, 20 of the 23 patients no longer required insulin and 12 continue this status to present. The patients were followed-up for 14–52 months. HbA_{1c} levels were normal, and C-peptide levels (0.8 nmol/L before transplantation and 2.9 nmol/L after 3 years of follow-up) steadily increased, indicating that β-cell function normalised. Eight patients became transiently insulin free (6–47 months). Despite the inability to regulate their glucose levels with small doses of insulin, patients exhibited a sufficient increase in C-peptide levels (0.6 nmol/L before transplantation and 1.7 nmol/L after 4 years of follow-up) [41, 42]. The side effects of autologous transplantation included nausea, vomiting, febrile neutropenia and 2 cases of nosocomial pneumonia. There were no fatalities. Long-term complications included a case each of autoimmune hypothyroidism, diffuse toxic goitre and hypergonadotropic hypogonadism and 9 cases of transient oligospermia.

It is particularly important to note that no other cell therapy option (including IST and immunomodulators) except for autologous transplantation of HSCs results in a long-lasting insulin-free status. These results were recently been confirmed by a number of studies conducted at other medical centres. In a study conducted at the Medical University of Warsaw, a similar regimen for autologous transplantation of HSCs of 8 patients with early T1DM led to long-lasting insulin independence in all, and 7 maintained insulin-free status for an average of 6 months after transplantation [43]. Autologous transplantation of HSCs in patients with T1DM without ketoacidosis resulted in insulin independence in 70% cases, as reported by a research group from China [44].

In April 2009, an international randomised trial of autologous transplantation of HSCs during early-stage T1DM was initiated at the University of Chicago Medical Center. The outcome promises to facilitate the efficacy and safety assessment of autologous transplantation of HSCs in patients with DM.

CONCLUSIONS

Embryonic SCs and adult tissue SCs may serve as potential sources of insulin-producing cells for allogeneic transplantation [45–47] to treat T1DM and T2DM. However, this application is limited by the complexity of the immune system that recognises and destroys foreign cells, which in turn, are targeted to restore carbohydrate metabolism. Even allogeneic embryonic SCs may suffer from this fate [48]. To overcome this barrier, several different approaches have been proposed, aimed mainly at modulating the immune response of patients with DM [49]. However, a suitable solution is not available.

The application of autologous SC-derived insulin-producing cells appears to be potentially attractive and may help to overcome the major problems that complicate cell therapy in DM (such as immune rejection and deficit of suitable donors). Various types of autologous SCs can be derived from peripheral blood, umbilical cord blood, PG and other tissues.

The results of studies focused on pancreatic pluripotent SCs are interesting, although further studies are required to determine whether patients with DM have such cells and how they can be stimulated to differentiate in vivo. It is also necessary to determine the mechanisms responsible for the reverse differentiation of exocrine cells to exploit them for therapeutic applications. Reprogramming of differentiated exocrine cells into β-like cells in vivo using a combination of the 3 transcription factors NEUROG3, PDX1 and MAFA is of particular interest. These cells are similar to β-cells in terms of size, shape and ultrastructure and are also capable of secreting insulin. It should be possible to create cells capable of direct transdifferentiation without first transitioning through a pluripotent state. Inducing pluripotent SCs may provide an alternative to isolating and then programming the further differentiation of pancreatic pluripotent SCs. Most ongoing studies are aimed at transdifferentiation of cells in vitro. The search for pluripotent SCs in vivo and development of mechanisms for stimulating their differentiation should be productive. If not, the induction of pluripotent SCs may be useful for generating insulin-producing β-like cells. However, the specific mechanisms and factors responsible for the transdifferentiation of exocrine cells to pluripotent cells must be identified.

The findings for liver cells are more promising and less contradictory than those for pancreatic cells. Because liver cells are available for biopsy and can regenerate, they may provide a source of differentiated cells for autologous transplantation. However, a procedure must be developed for culturing differentiated liver cells in vitro as well as for safely inducing in vivo transdifferentiation in humans.

iPSCs provide another source of β-cells. Reprogrammed cells differentiate into insulin-producing β-like cells. The application of chemicals as well as adenoviral and episomal vectors to reprogramme diverse cell types into IPSCs may be more suitable for clinical use, because these methods circumvent the activation of human proto-oncogenes by viral genomes that can irreversibly integrate into the human genome.

Devising treatments based on pluripotent SCs obtained from the umbilical cord blood is attractive, because it appears to be relatively safe and SCs are not administered to the recipient directly, thus excluding teratogenic effects. Moreover, IST is not required, the cells exert an immunomodulatory effect and umbilical cord blood banks may provide a sufficient source of multipotent SCs. However, the efficacy of this therapy in humans is limited and does not lead to insulin-independence. Therefore, further study is required to optimise insulin-producing cell recovery mechanisms and immunomodulation.

DOI: 10.14341/DM2014220-28

The existence of insulin-producing SCs in peripheral blood is of particular interest. In animal models, transplantation of PB-IPCs in vivo induces insulin production and homing of these cells into islets, indicating that they act as progenitors of β-cells. These cells can be isolated and cultured in sufficient quantities. Furthermore, administration of PB-IPCs is safe, does not induce immune rejection and does not raise ethical issues. Optimisation of this treatment by inducing β-cell proliferation [e.g. with glucagon-like peptide (GLP-1)] may significantly enhance its potential. Further studies will help to develop effective approaches for autologous transplantation of PB-IPCs to treat patients with DM.

Despite the impressive clinical results using high-dose IST and autologous transplantation of HSCs in T1DM diagnosed early (insulin independence and restoration of β-cell function indicated by the levels of C-peptide), this therapeutic approach has certain limitations. The risk of infection after transplantation remains very high. The procedure is quite expensive and requires highly trained personnel such as haematologists, transfusiologist and emergency physicians. Further, the requirement for transplantation of HSCs during the 6 weeks after diagnosis may also complicate the selection of patients.

Although autologous transplantation does not induce immune rejection of a transplant, which is the main disadvantage of allogeneic transplantation, but the problem of autoimmunity associated with T1DM remains unsolved. Therefore, to achieve better results, it is necessary to study the possibility of combining this method with immunotherapy [50, 51]. Such an approach may restore the β-cell pool and prevent or mitigate autoimmunity or both.

For these reasons, cell therapy is among the most rapidly developing areas in clinical and experimental medicine. High expectations for achieving long-term remission and recovery from DM are associated with cell, transplantation and molecular genetic technologies.

DISCLOSURE INFORMATION

The authors declare no explicit or potential conflicts of interest with respect to the publication of this article.

REFERENCES

1. Дедов ИИ, Шестакова МВ. Сахарный диабет. Руководство для врачей. М; 2003. 455 с.[Dedov II, Shestakova MV. Diabetes mellitus. Rukovodstvo dlya vrachey. Moscow; 2003. 455 p.]
2. Vardanyan M, Parkin E, Gruessner C, Rodriguez Rilo HL. Pancreas vs. islet transplantation: a call on the future. Current Opinion in Organ Transplantation. 2010;15(1):124–130. DOI: http://dx.doi.org/10.1097/MOT.0b013e32833553f8
3. Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery. 1967;61(6):827–837. PubMed PMID: 5338113.
4. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR). Clin Transpl. 2008:45–56.
5. Lauria MW, Figueiró JM, Machado LJC, Sanches MD, Nascimento GF, Lana MQ, et al. Metabolic Long-Term Follow-Up of Functioning Simultaneous Pancreas-Kidney Transplantation Versus Pancreas Transplantation Alone: Insights and Limitations. Transplantation. 2010;89(1):83–87. DOI: http://dx.doi.org/10.1097/TP.0b013e3181bd0f83
6. White SA, Shaw JA, Sutherland DER. Pancreas transplantation. The Lancet. 2009;373(9677):1808–1817. DOI: http://dx.doi.org/10.1016/S0140-6736(09)60609-7
7. Scharp DW, Lacy P. Insulin independence after islet transplantation into type 1 diabetic patient. Diabetes. 1990;39(4):515–518. DOI: http://dx.doi.org/10.2337/diab.39.4.515
8. Robertson RP. Islet transplantation as a treatment for diabetes – a work in progress. N Engl J Med. 2004;350(7):694–705. DOI: http://dx.doi.org/10.1056/NEJMra032425
9. Zhao Y, Jiang Z, Zhao T, Ye M, Hu C, Yin Z, et al. Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells. BMC Med. 2012;10(1):3–10. DOI: http://dx.doi.org/10.1186/1741-7015-10-3
10. Bonnerweir S. Life and Death of the Pancreatic β Cells. Trends in Endocrinology and Metabolism 2000;11(9):375–378. DOI: http://dx.doi.org/10.1016/S1043-2760(00)00305-2
11. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429(6987):41–46. DOI: http://dx.doi.org/10.1038/nature02520
12. Петеркова ВА, Лаптев ДН. Перспективы терапии, направленной на восстановление пула β-клеток, при сахарном диабете. Сахарный диабет. 2009;(3):6–9. [Peterkova V, Laptev D. Prospects for therapy aimed at restoring the beta-cell pool in patients with diabetes mellitus (review of the literature). Diabetes mellitus. 2009; (3): 4–9.] DOI: http://dx.doi.org/10.14341/2072-0351-5444
13. Wang RN, Kloppel G, Bouwens L. Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia. 1995;38(12):1405–1411. DOI: http://dx.doi.org/10.1007/BF00400600
14. Gmyr V, Kerr-Conte J, Belaich S, Vandewalle B, Leteurtre E, Vantyghem MC, et al. Adult human cytokeratin 19-positive cells reexpress insulin promoter factor 1 in vitro: further evidence for pluripotent pancreatic stem cells in humans. Diabetes. 2000;49(10):1671–1680. DOI: http://dx.doi.org/10.2337/diabetes.49.10.1671
15. Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet. 2011;43(1):34–41. DOI: http://dx.doi.org/10.1038/ng.722
16. Solar M, Cardalda C, Houbracken I, Martín M, Maestro MA, De Medts N, et al. Pancreatic Exocrine Duct Cells Give Rise to Insulin-Producing β Cells during Embryogenesis but Not after Birth. Developmental Cell. 2009;17(6):849–860. DOI: http://dx.doi.org/10.1016/j.devcel.2009.11.003
17. Xu X, D' Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, et al. Beta cells can be generated from endogenous progenitors in

injured adult mouse pancreas. Cell. 2008;132(2):197–207. DOI: http://dx.doi.org/10.1016/j.cell.2007.12.015
18. Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and regeneration of adult beta-cells does not involve specialized progenitors. Developmental Cell. 2007;12(5):817–826. DOI: http://dx.doi.org/10.1016/j.devcel.2007.04.011
19. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–676. DOI: http://dx.doi.org/10.1016/j.cell.2006.07.024
20. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–872. DOI: http://dx.doi.org/10.1016/j.cell.2007.11.019
21. Stadtfeld M, Hochedlinger K. Induced pluripotency: history, mechanisms, and applications. Genes & Development. 2010;24(20):2239–2263. DOI: http://dx.doi.org/10.1101/gad.1963910
22. Tateishi K, He J, Taranova O, Liang G, D' Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. Journal of Biological Chemistry. 2008;283(46):31601–31607. DOI: http://dx.doi.org/10.1074/jbc.M80659720
23. Alipio Z, Liao W, Roemer EJ, Waner M, Fink LM, Ward DC, et al. Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic – like cells. Proceedings of the National Academy of Sciences. 2010;107(30):13426–13431. DOI: http://dx.doi.org/10.1073/pnas.1007884107
24. Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. Proceedings of the National Academy of Sciences. 2009;106(37):15768–15773. DOI: http://dx.doi.org/10.1073/pnas.0906894106
25. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic Memory and Preferential Lineage-Specific Differentiation in Induced Pluripotent Stem Cells Derived from Human Pancreatic Islet Beta Cells. Cell Stem Cell. 2011;9(1):17–23. DOI: http://dx.doi.org/10.1016/j.stem.2011.06.007
26. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced Pluripotent Stem Cells Generated Without Viral Integration. Science. 2008;322(5903):945–949. DOI: http://dx.doi.org/10.1126/science.1162494
27. Golestaneh N, Kokkinaki M, Pant D, Jiang J, DeStefano D, Fernandez-Bueno C, et al. Pluripotent Stem Cells Derived From Adult Human Testes. Stem Cells and Development 2009;18(8):1115–1125. DOI: http://dx.doi.org/10.1089/scd.2008.0347.
28. Zhu S, Li W, Zhou H, Wei W, Ambasudhan R, Lin T, et al. Reprogramming of Human Primary Somatic Cells by OCT4 and Chemical Compounds. Cell Stem Cell. 2010;7(6):651–655. DOI: http://dx.doi.org/10.1016/j.stem.2010.11.015
29. Ber I, Shternhall K, Perl S, Ohanuna Z, Goldberg I, Barshack I, et al. Functional, Persistent, and Extended Liver to Pancreas Transdifferentiation. Journal of Biological Chemistry. 2003;278(34):31950–31957. DOI: http://dx.doi.org/10.1074/jbc.M303127200
30. Yang L. Liver stem cell-derived β-cell surrogates for treatment of type 1 diabetes. Autoimmunity Reviews. 2006;5(6):409–413. DOI: http://dx.doi.org/10.1016/j.autrev.2005.10.009
31. Jones P, Courtney M, Burns C, Persaud S. Cell-based treatments for diabetes. Drug Discovery Today. 2008;13(19–20):888–893. DOI: http://dx.doi.org/10.1016/j.drudis.2008.06.014
32. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. Br J Haematol. 2009;147(2):262–274. DOI: http://dx.doi.org/10.1111/j.1365-2141.2009.07883.x
33. Haller MJ, Wasserfall CH, Hulme MA, Cintron M, Brusko TM, McGrail KM, et al. Autologous Umbilical Cord Blood Transfusion in Young Children With Type 1 Diabetes Fails to Preserve C-Peptide. Diabetes Care. 2011;34(12):2567–2569. DOI: http://dx.doi.org/10.2337/dc11-1406
34. Zhao Y. Stem Cell Educator Therapy and Induction of Immune Balance. Curr Diab Rep. 2012;12(5):517–523. DOI: http://dx.doi.org/10.1007/s11892-012-0308-1
35. Zhao Y, Wang YH, Mazzone T. Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. Exp. Cell Res. 2006;312(13):2454–2464. DOI: http://dx.doi.org/10.1016/j.yexcr.2006.04.008
36. Gaber AO, Fraga D, Kotb M, Lo A, Sabek O, Latif K. Human islet graft function in NOD-SCID mice predicts clinical response in islet transplant recipients. Transplantation Proceedings. 2004;36(4):1108–1110. DOI: http://dx.doi.org/10.1016/j.transproceed.2004.04.055
37. Kayali AG. The stromal cell-derived factor-1 /CXCR4 ligand-receptor axis is critical for progenitor survival and migration in the pancreas. The Journal of Cell Biology. 2003;163(4):859–869. DOI: http://dx.doi.org/10.1083/jcb.200304153
38. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in auto-immune disease: a consensus report written on behalf of the European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT. Bone Marrow Transplant. 1997;19(7):643–645. DOI: http://dx.doi.org/10.1038/sj.bmt.1700727
39. Openshaw H. High-dose immunosuppression and hematopoietic stem cell transplantation in autoimmune disease: Clinical review. Biology of Blood and Marrow Transplantation. 2002;8(5):233–248. DOI: http://dx.doi.org/10.1053/bbmt.2002.v8.pm12064360
40. Voltarelli JC, Couri C. Stem cell transplantation for type 1 diabetes mellitus. Diabetol Metab Syndr. 2009;1(1):4–4. DOI: http://dx.doi.org/10.1186/1758-5996-1-4
41. Couri CEB, Oliveira MCB, Stracieri ABPL, Moraes DA, Pieroni F, Barros GMN, et al. C-Peptide Levels and Insulin Independence Following Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus. JAMA. 2009;301(15):1573–1579. DOI: http://dx.doi.org/10.1001/jama.2009.470.
42. Snarski E, Milczarczyk A, Torosian T, Paluszewska M, Urbanowska E, Król M, et al. Independence of exogenous insulin following immunoablation and stem cell reconstitution in newly diagnosed diabetes type I. Bone Marrow Transplant. 2011;46(4):562–566. DOI: http://dx.doi.org/10.1038/bmt.2010.147
43. Li L, Shen S, Ouyang J, Hu Y, Hu L, Cui W, et al. Autologous Hematopoietic Stem Cell Transplantation Modulates Immunocompetent Cells and Improves β-Cell Function in Chinese Patients with New Onset of Type 1 Diabetes. The Journal of Clinical Endocrinology & Metabolism 2012;97(5):1729–1736. DOI: http://dx.doi.org/10.1210/jc.2011-2188
44. Hussain MA, Theise ND. Stem-cell therapy for diabetes mellitus. Lancet. 2004;364(9429):203–205. DOI: http://dx.doi.org/10.1016/S0140-6736(04)16635-X
45. Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM. Stem cells. The Lancet 2005;366(9485):592–602. DOI: http://dx.doi.org/10.1016/S0140-6736(05)66879-1

46. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells.. Nat Biotechnol. 2006;24(11):1392–1401. DOI: http://dx.doi.org/10.1038/nbt1259
47. Bradley JA, Bolton EM, Pedersen RA. Stem cell medicine encounters the immune system. Nat Rev Immunol. 2002;2(11):859–871. DOI: http://dx.doi.org/10.1038/nri934
48. Melton DA, Daley GQ, Jennings CG. Altered nuclear transfer in stem-cell research–a flawed proposal. N. Engl. J. Med. 2004;351:2791–2792. DOI: http://dx.doi.org/10.1056/NEJMp048348
49. Лаптев ДН. Иммунотерапия сахарного диабета 1 типа: современное состояние проблемы и ее перспективы (часть1). Проблемы эндокринологии. 2009;4:24–33. [Laptev DN. Immunotherapy for type 1 diabetes: state-of-the-art and prospects. Part 1. Problemy Endokrinologii. 2009;55(4):24–34.] DOI: http://dx.doi.org/10.14341/probl200955424-34
50. Лаптев ДН. Иммунотерапия сахарного диабета 1 типа: современное состояние проблемы и ее перспективы (часть 2). Проблемы эндокринологии. 2009;5:31–38.[Laptev DN. Immunotherapy of type 1 diabetes mellitus: current state-of-the-art and prospects. Part 2. Problemy Endokrinologii. 2009;55(5):31–38.] DOI: http://dx.doi.org/10.14341/probl200955531-38

INFORMATION ABOUT THE AUTHORS

Ivan I. Dedov	MD, PhD, Professor, Academician at the Russian Academy of Sciences, Director of the Endocrinology Research Centre, Moscow, Russian Federation
Igor' A. Lisukov	MD, PhD, Professor, Director of the Centre for Oncohaemotology and Transplantology, Northwestern State Medical University named after I.I. Mechnikov, St. Petersburg, Russian Federation
Dmitry N. Laptev	MD, PhD, Senior Researcher at the Paediatric Endocrinology Institute, Endocrinology Research Centre, 11, Dmitriya Ul'yanova street, Moscow, Russian Federation, 117036 **E-mail: laptevdn@ya.ru**