

TOPICAL REVIEW

Diabetes – adult stem cells as an future alternative therapy?

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Abstract: In both types of the diabetes mellitus, the lack of functional β -cells is crucial, leading to complications associated with development of hyperglycaemia. One way to achieve a constant normoglycemic state without hypoglycemic episodes is either whole pancreas transplantation, or transplantation of isolated islets of Langerhans. Another approach to correct the β -cell deficit is the stimulation of β -cells in pancreas to regeneration. The development of new diabetes therapy is the main goal for many scientists around the world. This article is focused on the stem cells and their potential for clinical applications (Ref. 47). Full Text (Free, PDF) [www. bmj.sk](http://www.bmj.sk).

Key words: diabetes, therapy, adult stem cells.

Diabetes is a complex metabolic disorder, manifested by hyperglycaemia, glycosuria, increased catabolism of proteins and ketoacidosis. Diabetes is the syndrome involving etiologically and clinically heterogeneous group of pathological conditions. The common symptom is persistent hyperglycaemia resulting from absolute or relative lack of insulin, respectively it's lack of action in target tissues. The deficit of insulin caused the problems of carbohydrates metabolism, associated with the development of metabolic disorders of fat and proteins as well as the impaired management of water and electrolyte balance. In the long persistence of diabetes arise chronic complications, of which the most common and most significant are degenerative changes in blood vessels and nervous system (1). According to the mechanism of origin, two types of diabetes are recognized.

Type I diabetes mellitus is an autoimmune disease (DM I), which is the second most common disease in childhood and has a long-term impact on the patient's entire organism. Over 6 % of the human population is affected worldwide (2). Type I diabetes is caused by a cell-mediated autoimmune destruction of pancreatic β -cells (3) by autoreactive T-lymphocyte clones, impairing the insulin production. The primary reason starting this autoimmune reaction is still unknown, but it is assumed, that several genetic and environmental factors are involved. In the time of clinical symptoms, the autoimmune process is markedly advanced. Usually 60–80 % of the β -cells have been destroyed at

the time of diagnosis (4). There are currently 18 defined regions of the human genome, which have found to be in an association with the development of DM 1A and many another are presumed. The most significant genetic risk factor is the HLA gene complex localized on chromosome 6p21. Associations of HLA class II loci, allelic variants with T1 DM, are well established. Another prime candidate, particularly the polymorphic DPB1 gene, has been reported as probably contributing to the disorder, but its relative contribution to the predisposition to the disease is difficult to assess due to strong linkage disequilibrium of HLA alleles (5).

Standard therapeutic approach

Since 1920's, insulin therapy has changed diabetes from a rapidly fatal disease to a chronic disease associated with significant secondary complications, such as retinopathy, neuropathy, cardiovascular disease and renal failure. It is demonstrated that the risk of diabetic complications depends on the degree of glycemic control of the patients. The long-term studies have shown that strict monitoring of glycaemia with conventional or intensive insulin therapy, self blood glucose monitoring and patient education can significantly prevent and inhibit the progress of complications of this chronic disease (6–8). Insulin therapy that maintain glucose levels close to normal values and reduces the risk of secondary complications is by far the most common and most popular. Nevertheless, patients consider this treatment difficult and are exposed to increased risk of hypoglycaemia (9). This is caused by the fact that external insulin injection can not mimic the physiological control that pancreatic β -cell-derived insulin secretion exerts on the body's glycaemia.

Stem cells – biology and characteristic

Stem cells are characterized as undifferentiated cells which have been derived from embryonic, foetal and adult organisms

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(10–12). These cells are capable of self-renewing and they are unique in their potential to generate various types of tissues under proper conditions *in vitro* and *in vivo* (13).

Embryonic stem cells are considered to be pluripotent, i.e. they can create virtually all types of cells in human body. But their utilization is restricted by ethical problems considering the way how they are obtained, as well as higher risk of malignancy (14).

Over the past few years, adult stem cells have (ASC) been derived from various types of tissues including bone marrow, umbilical cord blood, adipose tissue, skin, periosteum, dental pulp, etc. Adult stem cells are heterogeneous population of cells and expressing also a variety of surface markers including CD29, CD44, CD90, CD105, STRO-1 and Sca-1. Moreover, they are negative for haematopoietic markers CD34, CD45 and for HLA Class II (15, 16). ASC are adherent and have a fibroblast-like morphology when cultured *in vitro*. No ethical problem is associated with their acquisition and there is also a low risk of malignancy. For this reason, multipotent adult stem cells are a promising tool for the new clinical field of regenerative medicine and tissue engineering.

Stem and progenitor cells in pancreas and neogenesis

Mature pancreas posses two functional parts: the exocrine part (99 %), includes acinar and ductal cells and endocrine part (1 %) containing islets of Langerhans. The islets are formed by four types of cells that are synthesising and excreting peptide hormones: β -cells (insulin), α -cells (glucagon), δ -cells (somatostatin) and PP-cells (pancreatic polypeptide) (17). Until now it was assumed that the final number of pancreatic β -cells is determined at birth. During embryonic development, cells in the pancreatic anlage migrate from the ducts while differentiating to form clusters that will become islets. So the post-natal pancreatic duct may harbour islet stem/precursor cells (18). It has been in reality described that adult rat and human islets of Langerhans contain nestin-positive progenitor cells, which can be differentiated into insulin-expressing cells *ex vivo* (17, 19, 20). Also ductal tissue from human pancreas was expanded and differentiated into functional islets tissue *in vitro* (21). Pancreatic duct cells act as progenitors, giving rise to new islets after birth and after injury.

On this basis, a theory has been developed, where the regeneration of pancreas can occur in two ways: by replication of pre-existing endocrine or exocrine cells from remaining non-damaged part of pancreas, or by proliferation and differentiation of ductal stem cells (22).

The mechanism of islet regeneration remains poorly understood. For the better understanding of β -cell regeneration, the identification and isolation of islet progenitors is crucial (23).

Beta cell preservation is also an important target in the treatment of type 1 DM and in prevention of its related complication (24). Many clinical trials have evaluated the role of immunointervention in preventing residual beta cell loss by blocking the autoimmune response with prednisone, azathioprine, cyclosporine, antibodies against CD3, heat shock protein and rabbit antithymocyte globulin. These therapies were shown to induce a slo-

wly decline or some improvement in C-peptide levels when compared with placebo groups. After stopping the immunosuppression, these effects were not maintained and almost all patients required again the exogenous insulin use (25–33).

Stem cells in diabetes therapy

Accessible cell therapy of diabetes is based mainly on the transplantation of islets or whole pancreas. There is a major progress on islet transplantation, which includes substantial improvements in islet isolation technology offering viable and functionally intact islets. Transplants entail a number of potential complications such as diabetogenic effect of some immunosuppression, immune rejection and oncogenic effect of long-term immunosuppressive therapy (34, 35). The remaining problem is the lack of suitable organs and availability of human islets from cadaveric pancreata is also limited (36).

An alternative to the transplantations of organs or tissues could be adult stem cells with their potential for developing into pancreatic beta-cells. They can adopt a pancreatic endocrine phenotype and they appear to be the best prospect for overcoming the islet shortage. Regeneration of pancreatic beta cells from somatic stem cells may overcome the limited source of islets and transplant rejection if beta cells are regenerated from endogenous – autologous adult stem cells. Procedures that reduce *in vitro* manipulation of cells and allow stem cells to develop into islets *in vivo* are crucial. It was demonstrated that ASC from different sources (bone marrow, adipose tissue, umbilical cord blood) are able to differentiate to functional insulin-producing cells (IPCs) (3). Creation IPCs from ASC represents an attractive alternative to pancreas transplantation.

ASC are also able to induce angiogenesis both, *in vivo* (37) and *in vitro* (38), but the mechanism is unclear. Oswald et al differentiated stem cells from bone marrow to cells with phenotypic and functional characteristics of endothelial cells (39). Kinnaird et al. demonstrated that ASC can secrete a large number of arteriogenic and angiogenic cytokines, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Angiopoietin-1 (Ang-1), matrix metalloproteinase (MMPs) and transforming growth factor- β (TGF- β) (40).

Experimental models and clinical trials

Bone marrow is a rich source of easily available adult stem cells, that can become parenchymal cells after entering liver, intestine, skin, lung, skeletal muscle, heart muscle and central nervous system in rodent models and in human recipients of marrow or organ transplantation (41, 42).

Ianus et al reported that mouse bone marrow-derived cells can differentiate into pancreatic β -cells with insulin secretion when transplanted into lethally irradiated mice (43). Another group showed that transplantation of c-kit positive mouse bone marrow-derived stem cells initiated endogenous pancreatic regeneration and improved blood glucose level in streptozotocin (STZ) – induced diabetic mice (44). Urbán et al used also an

animal model with STZ-induced diabetes. They used co-transplantation of bone marrow cells (BMCs) and syngenic or allogenic mesenchymal stem cells (MSCs). After administrated into sublethally irradiated diabetic mice blood glucose and serum insulin concentrations rapidly returned to normal levels, accompanied by efficient tissue regeneration after a single injection of cells. Researchers suggested two aspects of this successful treatment. First BMCs and MSCs induce the regeneration of recipient-derived pancreatic insulin-secreting cells. Second, MSCs inhibit T-cell-mediated immune responses against newly formed β -cells (45). Lee et al studied the survival and function of encapsulated human β -cells and their progenitors and the engraftment of encapsulated murine β -cells in allo- and autoimmune settings. Human islets and human fetal pancreatic islet-like cell clusters were encapsulated in polytetrafluorethylene devices (TheraCyte) and transplanted into immunodeficient mice. Encapsulated human islet-like cell clusters survived, replicated resulting in robust long-term allograft survival, and acquired a level of glucose responsive insulin secretion sufficient to ameliorate hyperglycemia in diabetic mice (46).

If rodents bone marrow cells can differentiate into functional pancreatic endocrine cells, bone marrow transplantation could be a prospective therapy of autoimmune type I diabetes, even though about the fate of transplanted cells, leads extensive discussion. Additionally it is difficult to overcome the persisting hostile beta cell-specific autoimmune response that may destroy the regenerated beta cells (36).

Saudek et al initiated a prospective randomized controlled trial of polyclonal anti-T-cell globulin (ATG) in patients with type 1 diabetes. During the first 12 months, a significant difference in the insulin dose and in the glucagon stimulated C-peptide level was found. They suggest that a short-term ATG therapy in Diabetes type 1 contribute to preservation of residual C-peptide production and to lower insulin requirements in the first year following diagnosis (33).

Voltarelli et al in a prospective study transplanted the autologous nonmyeloablative hematopoietic stem cells (HSCs) in newly diagnosed type 1 DM. HSCs were mobilized, then collected from peripheral blood by leukapheresis and cryopreserved. The cells were injected intravenously after conditioning with cyclophosphamide and rabbit antithymocyte globulin. During 7- to 35 month follow-up, 14 patients (from 15) became insulin-free. After high-dose immunosuppression and autologous nonmyeloablative HSCs transplantation, beta-cell function was increased and induced prolonged insulin independence in majority of the patients (47).

Conclusion

There is a solid evidence that insulin-producing cells may be derived from stem cells, but there is a need for further development of methods for differentiation and selection of completely functional β -cells. Depending on the regulation of various factors, β -cells can arise also by regeneration. Therefore, understanding this regulation is critical for the success in efforts to

treat diabetes. Also we cannot forget that type 1 diabetes is an autoimmune disease, and while the cell therapy may restore function, the mechanisms and factors that destroyed the original β -cells are still present. Therefore there is a necessity to find the best way how to affect the autoimmune process. There is no perfect solution for the cure of diabetes type 1 at the present time, but the research on a variety of potential approaches will in the near future offer the best choice for the cure of human diabetes type 1.

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