STEM CELLS TECHNOLOGY: A TOOL FOR TREATMENT OF INSULIN DEFICIENCY IN HUMAN

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ABSTRACT

Undifferentiated cell capable of self-renewal and differentiate into more specialized cells. They have a unique ability to give rise to many different cell types such as skin, liver, kidney, heart, neuron or other organ cells. Therefore also called "body's master cells". Since diabetes is a disease with a major deficiency in the functioning of one type of cell, there is potential of stem cells to treat type 1 diabetes and to improve the quality of life for those with type 2 diabetes. As researchers learn more about the mechanisms that govern stem cell programming, differentiation, and renewal, their ability to identify, isolate, and culture candidate stem cells will continue to improve. Clearly, using stem cells to treat diabetes will require better alternative sources using which, though stem cells can be currently considered a frontier for diabetes therapy, they may one day become its basis.

Key words: Undifferentiated cell, body's master cells, type 1 diabetes, stem cell

INTRODUCTION

Current treatments for type II diabetes are based on chemical methods that increase the body's tissue sensitivity to insulin; however, these therapies do not address the underlying causes of the disease, specifically, the pancreatic beta cell dysfunction and the insulin resistance. Additionally, secondary complications such as those mentioned above, namely, peripheral neuropathy, organ dysfunction and peripheral vascular disease, also are not improved by current approaches. In many cases current diabetes drug therapies do not provide sufficiently tight control of blood glucose to avoid diabetic late complications.[1, 2] Transplantation of whole donor pancreas is an effective form of treatment but is of limited application since it entails major surgery and long term immunosuppressant.

This failure to prevent the morbidity associated with diabetes places an enormous burden not only on patients and their relatives but also on society. The costs of treating late diabetic complications are set to escalate because of the predicted sharp rise in the number of people with diabetes. Thus, both patients and society have much to gain from development of improved treatment for diabetes. There is great interest in developing strategies to expand the population of functional b-cells. Possible ways to achieve this include physically replacing the b-cell mass via transplantation, increasing b-cell replication, decreasing b-cell death, and deriving new b-cells from appropriate progenitor cells.[3] In 1990, physicians at the Washington University Medical Center in St. Louis reported the first successful transplant of donor-supplied pancreatic islet tissue (which includes b-cells; see below) in humans with type 1 diabetes.[4] By the end of the decade, many other transplants had been reported using various protocols, including the widely-known "Edmonton protocol" (named for the islet transplantation researchers at the University of Alberta in Edmonton).[5-7] This protocol involves isolating islets from the cadaveric pancreatic tissue of multiple donors and infusing them into the recipient's portal vein. However, the lack of available appropriate donor tissue and the strenuous regimen of immunosuppressive drugs necessary to keep the body from rejecting the transplanted tissue limit the widespread use of this approach. Moreover, the isolation process for islets damages the transplantable tissue; as such, 2–3 donors are required to obtain the minimal b-cell mass sufficient for transplantation into a single recipient.[6] while these strategies continue to be improved, islet function declines relatively rapidly post-transplant. For example, a long-term follow-up study of Edmonton transplant patients indicated that less than 10% of recipients remained insulin-independent five years after transplant.[8]

Challenges: These challenges have led researchers to explore the use of stem cells a possible therapeutic option. Type 1 diabetes is an appropriate candidate disease for stem cell therapy, as the causative damage is localized to a particular cell type.

Properties of stem cells: They possess the property of self-duplicating for indefinite periods of times [9-10] Stem cells are capable of dividing and renewing themselves. They are unspecialized. They can give rise to specialized cell types.

Under certain physiologic or experimental conditions, they can be induced to become cells with special functions such as the beating cells of the heart muscle or the insulin-producing cells of the pancreas.

Types of Stem cells [11-20]

According to divisional capacity: A single **totipotent** stem cell can grow into an entire organism and even produce extra-embryonic tissues. Blastomeres have this property.

Pluripotent stem cells cannot grow into a whole organism, but they are able to differentiate into cells derived from any of the three germ layers.

Multipotent stem cells can only become some types of cells.[11]

Unipotent: - Produce only one cell type

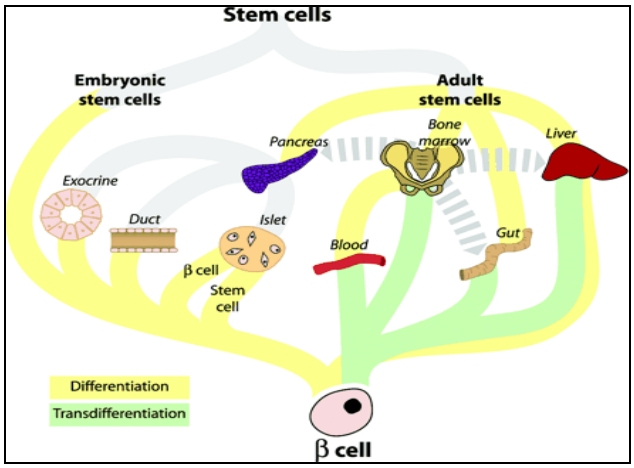

Figure1: Different routes from stem cells to β-cells.[21]

Types of Stem Cell Transplantation Process: Based on the source of stem cells: Bone marrow stem cell transplantation, Peripheral blood stem cell transplantation, Cord blood stem cell transplantation

Based on the donor: Autologous stem cell transplantation, allogeneic stem cell transplantation, syngeneic stem cell transplantation, Tandem autologous transplantation, Mini allogeneic transplantation.

In **Autologous stem cell transplantation**, *you are your own donor*. The stem cells come from either your bone marrow or your blood. Your stem cells are removed, or *harvested*, before treatment and then frozen. After you get high doses of chemo and/or radiation the stem cells are thawed and given back to you.

An **advantage** of autologous stem cell transplant is that you are getting your own cells back. This means there is no risk that your immune system will reject the transplant or that the transplanted cells will attack your body.

A possible **disadvantage** is that cancer cells may be harvested along with the stem cells and then put back into your body. To prevent this, doctors may use anti-cancer drugs or other therapies to treat your stem cells and reduce the number of cancer cells that may be present. This is called *purging*. Purging may damage some healthy stem cells, so extra cells are taken from the patient before the transplant to be sure that enough healthy stem cells will be left after purging.

Stem Cell Transplantation Process [22]

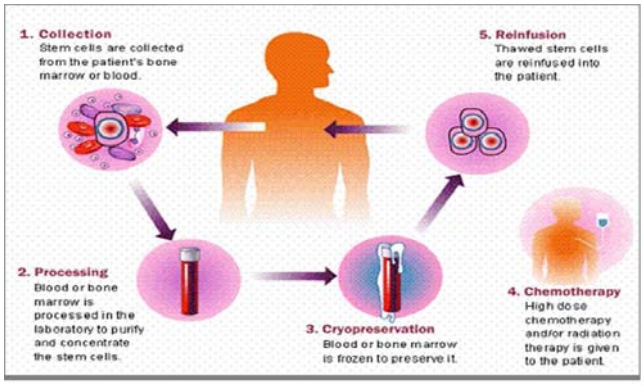

Figure 2: autologous stem cell transplantation

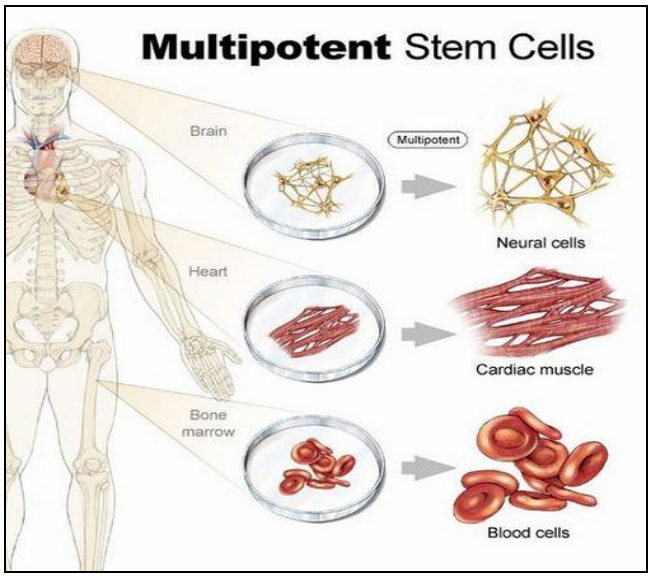

Figure 3: Multipotent stem cells

Collection: Most of the stem cells in the human body reside in the bone marrow. Until recently, the only way to obtain stem cells for transplantation was to remove a portion of the bone marrow. However, through recent medical advances, it is now possible to collect stem cells from a person's peripheral blood (that is, from the arm or another site outside the bone marrow). Today, most autologous transplants utilize peripheral blood stem cells (PBSCs). Bone marrow stem cells are more often used in allogeneic transplants, but PBSCs are beginning to be used more frequently.

Collecting stem cells from bone marrow*:* Collecting, or "harvesting," bone marrow is usually done in a hospital operating room under general anesthesia. Using a needle and syringe, a surgeon will take bone marrow from several different areas of the hip bone (pelvis). The bone marrow, which appears as a thick red liquid, is typically frozen and stored until high-dose chemotherapy is completed.

Collecting stem cells from peripheral blood: Harvesting stem cells from the blood takes approximately a week and has certain advantages over collecting stem cells from bone marrow. Because most stem cells reside in the bone marrow, it is necessary to move stem cells from the bone marrow to the bloodstream prior to their collection. This procedure is called mobilization. Once a sufficient number of stem cells are mobilized from the bone marrow into the bloodstream, the stem cells are collected using a non-surgical procedure called apheresis. **Processing and Cryopreservation:** After the bone marrow or peripheral blood stem cells are collected from the patient, they are processed in the laboratory, cryopreserved (frozen), and stored until needed. **High-dose Chemotherapy:** After the bone marrow or stem cells are collected, or at some later date, the patient will receive high-dose chemotherapy. The higher doses of chemotherapy are designed to destroy cells more effectively than standard chemotherapy. Some patients may receive one or more treatments of high-dose chemotherapy, *possibly in combination with radiation therapy, over a period of several days.*

Infusion:

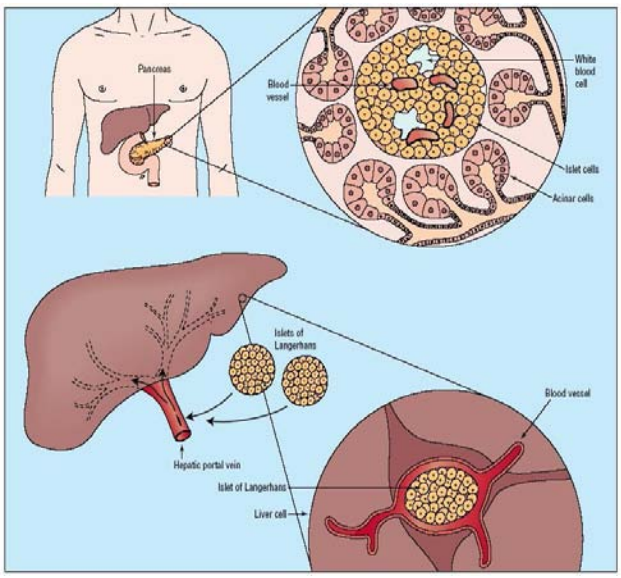

Figure 4: Islet cell transplant by injection into hepatic portal vein

The infusion process is similar to a blood transfusion, and can be done on an outpatient basis in some cases. The frozen bags of bone marrow or blood cells are thawed in a warm water bath and infused into a vein over a period of 2 to 4 hours. The infused stem cells travel through the bloodstream and settle in the bone marrow, where they begin to produce new white blood cells, red blood cells, and platelets.

Engraftment and Recovery: During the first few days after transplantation, the reinfused stem cells migrate to the bone marrow and begin the process of producing replacement blood cells, a process called engraftment. The stem cells start to produce new blood cells within 12 to 15 days following infusion. Colony-stimulating factors may be administered during this time to stimulate the process of blood cell production. Until engraftment is complete, a transplant recipient is susceptible to infection, anemia, and bleeding caused by low blood cell counts. Therefore, special precautions are necessary during recovery. Patients may be given red blood cell and platelet transfusions during the recovery period to help prevent anemia and bleeding. For the first 2-4 weeks after the transplant, patients are very susceptible to developing infections. This is because the effects of the high-dose chemotherapy and the loss of blood cells weaken the body's immune system. Antibiotics are often prescribed to help prevent infection.

Allogenic Stem Cell Transplantation

Here, the stem cells do not come from the patient, but from a donor whose tissue type (described later under "HLA matching") best matches the patient. The donor is most often a family member, usually a brother or sister. If you do not have a good match in the family, a donor may be found from the general public through a national registry. This may be called a MUD (matched unrelated donor) transplant. Blood taken from the placenta and umbilical cord of newborns is a newer source of stem cells. This small amount of blood has a high number of stem cells. But the numbers are often too low for large adults, so this source of stem cells is used mostly in small adults and children.

An **advantage** of allogeneic stem cell transplant is that the donor stem cells make their own immune cells, which may help destroy any cancer cells that may remain after high-dose treatment. Another possible advantage is that the donor can often be asked to donate more stem cells if needed. Stem cells from healthy donors are also free of cancer cells.

Syngeneic stem cell transplant This is a special kind of allogeneic transplant because the donor is an identical twin with identical tissue types. Since few people are identical twins, this type of transplant is very rare. An advantage of syngeneic stem cell transplant is that graft-versus-host disease will not be a problem. A disadvantage is that this type of transplant won't help destroy any remaining cancer cells. So every effort must be made to destroy all the cancer cells before the transplant is done.

Tandem transplants A tandem transplant is a "double autologous transplant." In a tandem transplant, a patient gets 2 courses of high-dose chemo, each followed by a transplant of their own stem cells. All of the stem cells needed are collected before the first high-dose chemo treatment, and half of them are used for each procedure. Most often both courses of chemo are given within 6 months, with the second one done after the patient recovers from the first one. Researchers hope that this method can keep the cancer from coming back and are still studying how this method can best be used.

Non-myeloablative or mini-transplants[23]Another type of allogeneic transplant is called a *mini-transplant*. It may also be called a *non-myeloablative transplant* or a *reduced-intensity transplant*. Another possibility that is being studied is autologous transplant followed by an allogeneic mini-transplant.

Potential Side-effects: Graft-versus-host disease, Stem cell (graft) failure, Organ damage, Blood vessel damage, Cataracts, Secondary cancers, Death

Stem cells derived from haemopoietic organs: Bone marrow harbours cells that can become parenchymal cells after entering the liver, intestine, skin, lung, skeletal muscle, heart muscle, and central nervous system,[24] in rodent models and in human recipients of marrow or organ transplantation.[25,26] In rodents, haemopoietic organs harbour cells that can also differentiate into functional pancreatic endocrine cells.[27-32]1–2 months after bone-marrow transplantation, donor derived cells are found in pancreatic islets of recipient mice. These cells express insulin and genetic markers of cells. In culture, the cells secrete insulin in response to glucose, and show intracellular calcium fluctuations similar to normal _ cells. However, only about 1–3% of the islet cells originate from the transplanted marrow (figure, A).[27]A marrow-derived cell-type with pluripotential capacity to transdifferentiate into various phenotypes has been described. [33]This or a similar cell type might be able to differentiate into pancreatic cells.

Similar experiments have been done in overtly diabetic mice whose _ cells have been destroyed by

streptozotocin. After bone-marrow transplantation, blood glucose and insulin concentrations were normal, and survival was better.[28] in islets, marrow-derived cells had differentiated into endothelial cells and occasionally into insulin expressing cells.[28] Endothelial engraftment was speculated to stimulate the proliferation of local pancreatic progenitors, leading in turn to the increased insulin producing cell-mass.

Pancreatic tissue showed increased proliferative activity and regeneration of cells. Thus marrow transplantation to induce immunological control plus maintenance of normoglycaemia allowed local or progenitor cells to proliferate as an adaptive response.[29] Transplantation of mesenchymal cells from the spleen combined with complete Freund's adjuvant led to reversal of diabetes accompanied by regeneration of insulin producing islets.[30]The transplanted splenic mesenchymal cells differentiate into cells. Thus splenic mesenchymal cells transplanted under certain conditions seem not only to keep immune destruction of islets in check, but also can transdifferentiate into pancreatic cells. The relative functions seemed to arise from different subpopulations.[31]

Bone-marrow cells can differentiate in vitro under controlled conditions into insulin-expressing cells [33, 34] such cells, transplanted under the kidney capsule of diabetic rodents, correct glucose. Removal of the grafted kidney returned the animals to a diabetic state.[35]

Cell fusion has been suggested as a mechanism of apparent adaption of bone-marrow-derived cells into an extramedullary phenotype. [32, 36] Studies involving pancreatic endocrine-cell differentiation from haemopoietic-organ derivatives largely rule out cell-fusion events as a mechanism of transdifferentiation. [27-31] Bone-marrow-derived extramedullary parenchymal tissue is not always found [36]. Some groups find little if any transdifferentiation [37, 38] bone-marrow derivation of intra-islet endothelial cells in diabetic mice.[39] The last finding was made recently by Andreas Lechner and colleagues. One group reports the generation of insulin-producing cells in liver, adipose tissue, spleen, and bone marrow in rodent models of diabetes mellitus.[40]Bone-marrow transplantation shows that most if not all extra pancreatic insulin-producing cells derive from donor bone-marrow.[40]

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Stem Cell Transplantation Process [38]

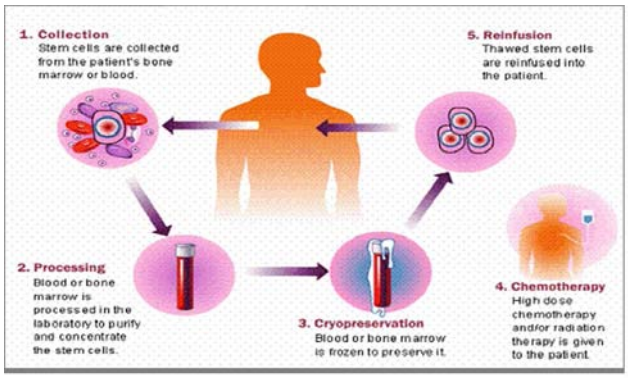

Figure 5: autologous stem cell transplantation

Collection: Most of the stem cells in the human body reside in the bone marrow. Until recently, the only way to obtain stem cells for transplantation was to remove a portion of the bone marrow. However, through recent medical advances, it is now possible to collect stem cells from a person's peripheral blood (that is, from the arm or another site outside the bone marrow). ***Collecting stem cells from bone marrow***. Collecting, or "harvesting," bone marrow is usually done in a hospital operating room under general anesthesia. Using a needle and syringe, a surgeon will take bone marrow from several different areas of the hip bone (pelvis). ***Collecting stem cells from peripheral blood***. Harvesting stem cells from the blood takes approximately a week and has certain advantages over collecting stem cells from bone marrow.

How adult stem cell therapy is an ideal treatment for diabetes[41]

Because it is the only therapy that addresses all the drawbacks of conventional treatment. Through *inhibiting the production of inflammatory mediators such as TNF-alpha*, adult stem cells contribute to *increasing insulin sensitivity*. The additional ability of adult stem cells to directly differentiate into beta cells, as well as to induce endogenous insulin secretion, has also been demonstrated in several scenarios. Furthermore, the regenerative ability of adult stem cells is not limited only to increasing endothelial health and organ function but also to decreasing neuropathic pain. Let's look at each of these properties individually.

Increasing insulin sensitivity with adult stem cells[41]:

The insulin receptor signaling pathway is very sensitive to inflammatory mediators. Specifically, cytokines such as TNF-alpha inactivate the insulin receptor through mechanisms such as phosphorylation of serine on the insulin receptor substrate. Clinical support for the role of TNF-alpha in insulin resistance comes from studies demonstrating positive correlations between this cytokine and the severity of type II diabetes. The primary source of TNF-alpha in type II diabetic patients is adipose tissue. For example, large volume liposuction has been demonstrated to temporarily reduce TNF-alpha levels as

well as the extent of insulin resistance. Various types of adult stem cells have demonstrated a highly potent ability to block the production of TNF-alpha, and it is also known that the blood-making compartment in the bone marrow is very sensitive to TNF-alpha. Accordingly, one of the natural functions of the mesenchymal stem cell, which resides in the bone marrow, is to inhibit production of this cytokine from other cells. Mesenchymal stem cells produce a variety of TNF-alpha inhibitor compounds such as IL-10 (interleukin 10) and TGF-beta. The potency of mesenchymal stem cells to shut down TNF production has therefore been well established by a number of researchers.

Thus by inhibiting TNF alpha and releasing numerous anti-inflammatory mediators, mesenchymal stem cells offer the possibility of decreasing insulin resistance by targeting the underlying inflammatory cause. Such anti-inflammatory activities of mesenchymal stem cells have also been demonstrated in other conditions associated with pathological immune activation.

Restoration of insulin production by adult stem cells:

Adult stem cells are known to differentiate into a variety of different cell types. The public media discusses at length the possibility of being able to use embryonic stem cells, perhaps, some day, at some distant time in the future, to generate new tissue, however, embryonic stem cells are well known to be tumor-causing, by definition, and they have never actually been used clinically, for this as well as numerous other inherent risks. In sharp contrast, adult stem cells such as bone marrow and cord blood stem cells, which have been administered to thousands of patients without adverse effects, are already recognized as being capable of differentiating into therapeutic cells such as insulin producing cells. The production of insulin has already been demonstrated in animal models in which mesenchymal stem cells were administered into mice whose beta cells had been damaged by the administration of the toxic compound streptozoacin, after which, increased insulin production was measured in the mice as a direct result of the mesenchymal stem cells.

The use of adult stem cells to induce islet regeneration is also currently undergoing U.S. FDA approved clinical trials at the University of Miami. Additionally, results from numerous clinical studies involving the administration of bone marrow stem cells by physicians outside of the U.S. have been very promising. For example, one group in Argentina has reported that 85% of Type II diabetic patients who were treated with their own mesenchymal stem cells were able to stop using insulin. The possibility of stimulating islet regeneration does not necessarily depend on differentiation of the adult stem cells into new islet cells but may also occur through the production of growth factors made by the stem cells and which allow endogenous pancreatic stem cells to start proliferating, thereby healing the injured area. For example, from a mouse study in which chemically labeled bone marrow stem cells were administered into mice with injured beta cells, the stem cells were actually found to stimulate the islet activating pancreatic duct stem cell proliferation. The possibility of stimulating endogenous pancreatic duct stem cells by pharmacological means is currently under investigation by the company Novo Nordisk, who has administered a combination of EGF and gastrin to diabetic patients in Phase II clinical trials. However, given that adult stem cells produce a "symphony of growth factors", including gastrin and EGF, the administration of stem cells seems to possess a higher possibility of success. Regardless of whether stem cells directly differentiate into pancreatic cells, or activate endogenous pancreatic stem cells, from the preclinical and clinical data available, there is strong evidence to indicate that these cells are therapeutic for the restoration of insulin production.

Alternative Sources of Stem Cells[42-52]

Sources	Scientific studies (In Vivo/ In Vitro study)
Embryonic stem cells	Generate embryoid bodies that contain cells with a B-cell-like phenotype with over expression of PAX4, PDX1 or NKX6. during differentiation in **human ESCs,** while in case of **Marine ESCs** over **expression of insulin.** **Failure of study** due to development of tumors **in animal.**
Bone-marrow derived Stem cells	BMSCs in regenerative processes **in vivo** remain the subject of study because infusion of bone-marrow cells can **restore chemically induced diabetes in mice**. Notably, however, it has not been indisputably shown that BMSCs differentiate into B-cells.
Placenta-derived multipotent stem cells	Differentiation into insulin-positive cells which functionally **secrete insulin in vitro Control blood glucose levels in vivo**
Liver stem cells	When transplanted into immunodeficient diabetic mice, transduced with human telomerase (hTERT) and then PDX-1 produced cells with considerable amounts of stored and secreted insulin and maintained euglycemia for prolonged periods.[51, 52]
Gastrointestinal adult stem cells	Transfected intestinal stem cells from the rat with genes encoding Pdx-1 and Isl-1, followed by exposure to the peptide betacellulin, which promotes Pancreatic B-cell differentiation. The resultant cells made insulin, and reduced glucose levels in vivo.
Adipose tissue derived stem cell	During the proliferation period, the cells expressed stem cell markers nesting, ABCG2, SCF, Thy-1 and Isl-1 together with induction of insulin, glucagon, and somatostatin.

Reversing secondary complications[41]:

Uncontrolled blood glucose levels are associated with a variety of complications such as peripheral vascular disease, neuropathic pain and the dysfunction of various organs, for example, renal failure. It is known that stem cell therapy can ameliorate, or in some cases, reverse these pathologies. Peripheral vascular disease, for example, is caused by endothelial dysfunction, but we also know that there is a constant migration of endothelial progenitors from bone marrow sources to the periphery. This migration can be measured through the quantification of the content of endothelial progenitor cells in peripheral blood, and in this manner it has been observed that patients who are diabetic and who have higher levels of circulating endothelial progenitors usually have a lower risk of coronary artery disease.

The administration of adult stem cells is known to rejuvenate old or dysfunctional endothelial cells, and to increase responsiveness to vasoactive stimuli.

Future: At the moment, it is difficult to know which source of stem cells has the greatest potential. The b-cell is a very complex and differentiated cell. Thus adult stem cells, particularly those coming from the pancreas, seem to be easier to fully differentiate in normal B-cell with precise glucose recognition and regulated insulin secretion. In contrast, embryonic stem cells are more difficult to differentiate, but proliferation is not a major problem. In addition, regardless of the origin of newly generated b-cells, these cells will need to function in other places besides the pancreas and will need to be protected from rejection and autoimmune destruction. Thus research on both adult and embryonic stem cells should be pursued, because embryonic stem cells will be crucial to improve the research in adult stem cells and vice versa.

CONCLUSION

From this review article we can conclude that, modulating the autoimmune response in type 1 diabetes remains a significant challenge regardless of the type of cell that is transplanted, and it will also be important to address the insulin resistance in type 2 diabetes, as well as factors that contribute to obesity. However, since diabetes is a disease with a major deficiency in the functioning of one type of cell, there is potential of stem cells to treat type 1 diabetes and to improve the quality of life for those with type 2 diabetes. As researchers learn more about the mechanisms that govern stem cell programming, differentiation, and renewal, their ability to identify, isolate, and culture candidate stem cells will continue to improve. Clearly, using stem cells to treat diabetes will require better alternative sources using which, though stem cells can be currently considered a frontier for diabetes therapy, they may one day become its basis.

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