Challenges and prospects for stem cell-based therapy in Type 1 diabetes

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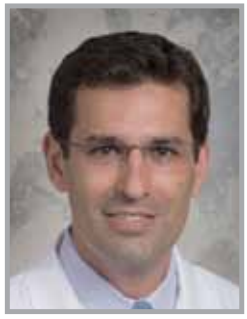

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Q Do you foresee greater potential for stem cell replacement therapy in Type 1 or Type 2 diabetes?

■ **Piemonti**

In both Type 1 and 2 diabetes, insufficient numbers of insulin-producing β cells are a major cause of defective control of blood glucose and its complications. Historical data indicate that between 67 and 90% of the original β-cell mass is destroyed in Type 1 diabetic patients at onset, while recent studies using hyperglycemic clamp and glucagon stimulation have confirmed a 75% deficit compared with healthy control patients. Similarly, Type 2 diabetes also presents a deficit in β-cell mass, which in

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later stages can be reduced by approximately 50% compared with a healthy individual. In principle, the treatment for Type 1 diabetes and many cases of Type 2 diabetes lies in the possibility of finding a β-cell mass substitute capable of performing two essential functions: assessing blood sugar levels and secreting appropriate levels of insulin in the vascular bed. Consequently, both types of diabetes can benefit from stem cell-derived insulin-producing cell.

Q Do you think stem cells will be a principal treatment for diabetes? If so, will you hazard a guess at when this will be the case?

■ **Piemonti**

It is not easy to answer this question. Currently, the only available clinical therapy capable of restoring β-cell mass in diabetic patients is the allogenic/autologous transplantation of β cells (somatic cell therapy with pancreas, Islets of Langerhans or β-cell transplantation). Despite advances in recent years, allogenic somatic therapy is still problematic (e.g., the need for immunosuppression therapy and, in the case of islet transplantation, the need for many donors for a single recipient, and the short life of the transplantation). This has led to increasing interest in alternative strategies. Recent investigations on the generation of insulin-producing cells has revealed that, in addition to primary source (i.e., pancreatic β cells), insulin-producing cells can be derived from several alternative sources, including embryonic, adult, mesenchymal and hematopoietic stem cells via the process of proliferation, dedifferentiation, neogenesis, nuclear reprogramming and transdifferentiation. Are these alternative sources really better than primary sources? This has not yet been proven. The field is rather controversial and difficult. Much information is still lacking on the molecular mechanisms and pathways of the signals controlling the expansion and differentiation of stem cells into insulin-producing β cells. Many results have been achieved in animal models and from cell lines, and it is still unclear whether primary human cells can be manipulated in the same manner. The generation of insulin-producing cells has not always been assessed with rigorous criteria and mere insulin expression is by no means adequate to determine a cell as being a β cell. The most effective protocols to date have produced cells that express insulin and have molecular characteristics that closely resemble *bona fide* insulin-secreting cells; however, these cells are often unresponsive to glucose. There are difficulties concerning *in vivo* human models that can allow us to assess the growth and proliferation of β cells and the control of carcinogenicity. Moreover, even if a sufficient number of functional stem cell-derived β cells will be available, unresolved problems, such as the identification of the best implantation site and/or the best strategy for tissue engraftment and survival, must be resolved. Finally, it should not be underestimated that both Type 1 and Type 2 diabetes mellitus are not characterized by acute damage to β cells (ischemia-like), which, while leading to the death of cells, vanishes over the course of time. Both insulin resistance and autoimmunity remain unvaried unless these too are therapeutically modified in therapeutic terms and any therapy with new β cells is destined to fail in the short term if it is not accompanied by the correction of the primary pathogenic noxa. We will need years to address all these issues.

■ **Domínguez-Bendala**

Unlike other conditions for which stem cell-based treatments have been foreseen, diabetes can now be treated with cell transplantation therapies. We know that long-term normoglycemia can be attained following islet transplantation, especially with the latest refinements in immunosuppresion techniques. In other words, the approach works. The main limitation remains the availability of insulin-producing cells, which is several orders of magnitude lower than the potential number of recipients. It is for this reason that we can safely assume that any future treatment for Type 1 diabetes will involve stem cell-derived islets.

Q What is the next single biggest challenge in the field?

■ **Piemonti**

Before *in vitro* stem cell-derived β cells can be used clinically, a number of impediments need to be surmounted. The first major challenge involves safety, including the tumorigenic potential of undifferentiated stem cells, which give rise to teratoma tumors. The second major challenge is the low efficiency of the differentiation process. Even with the improvements afforded by the recent studies (25% of cells expressing insulin) the issues of efficiency and functional maturity are important considerations that will need to be overcome before stem cell-derived β cells can be viewed as a viable therapeutic

option. We have to remember that a mass of 4.6×10^6 β cells/kg is needed to obtain insulin independence, as we learned from clinical protocols of islet transplantation.

■ **Domínguez-Bendala**

The next biggest challange is to establish the safety of embryonic stem (ES) cell-based treatments. Advances in differentiation methods have resulted in the generation of competent β-like cells that can efficiently correct hyperglycemia in diabetic animals. However, many of these techniques involve the transplantation of β-cell progenitors, which are allowed to mature upon transplantation. Carry-over undifferentiated cells are known to produce teratomas in a significant percentage of transplanted animals. Although it could be argued that such tumorigenic escapees might perish by simply removing immunosuppresion, few would dispute that this passive approach would not easily find its way to the clinic. In addition, as new autologous strategies with patient-derived induced pluripotent stem (iPS) cells progressively displace those based on ES cells, the immunosuppresion removal safeguard would no longer be applicable. Mechanical containment of teratomas, be it by encapsulation or the use of bioimplantable devices, may offer additional security. However, at the end of the day the challenge remains to prevent their appearance in the first place.

Q Is there any use for stem cell β-cell replacement therapy in Type 1 diabetes before scientists can control the autoimmune response?

■ **Piemonti**

To date, the use of stem cell β-cell replacement therapy in Type 1 diabetes has the same immunologic limitations of therapy with pancreatic islet transplantation. In fact, despite reports that stem cells and their differentiated progeny may be nonimmunogenic, recent studies have documented the development of immune responses against transplanted stem cell-derived tissues in immunocompetent mice. Therefore, at this time, patients receiving allogeneic stem cell-derived insulin-producing cells will require life-long immunosuppressive therapy. Furthermore, although the evolution of human cell technology can give rise to patient-specific insulin-producing cells, autologous transplantation in Type 1 diabetes will require the suppression of the pre-existing autoimmunity. In the future, the strategies to minimize or eliminate the requirement for immunosuppression under investigation for islet transplantation may also be applicable to stem cell β-cell replacement therapy (i.e., microencapsulation techniques, transplantation into immune-privileged sites and use of T regulatory cells to induce tolerance).

■ **Domínguez-Bendala**

The re-education (or resetting) of the immune system is the ultimate goal. However, if stem cell therapies are proven safe in the context of ever-evolving immunosuppressive protocols and/or immunoisolation devices, their use will still represent a major advance in patient care. Even if the grafts had a limited ability to survive beyond a few years, or even months, the inexhaustible supply of stem cells would make it possible to periodically replace batches of insulin-producing cells. This would offer patients a much better glycemic control than is currently attainable by any therapy other than islet or pancreas transplantation.

Q How far away are clinical trials of stem cell technology in diabetes?

■ **Piemonti**

Probably in the next 5 years a few pilot clinical studies will test the contribution of stem cells as a β-cell replacement via direct differentiation into insulin-producing cells. In fact, broadly speaking, stem cell technology in diabetes is already used in many clinical trials. Recently, the potential role of stem cells in β-cell regeneration has been reassessed from a different point of view. While direct differentiation is highly unlikely, a wide array of experimental evidence indicates that stem cells, in particular that of mesodermal origin (i.e., hematopoietic stem cells, mesenchymal stem cells [MSCs]) are capable of facilitating the survival, or the endogenous regeneration of, β cells through a regeneration process that is not well defined at present. Experimental *in vitro* and *in vivo* data associated with the easy availability of stem cells of mesodermal origin from bone marrow and wide consolidation of clinical experience in the field of hematology, has made it possible to design clinical trials on humans involving bone marrow-derived stem cell to cure both Type 1 or 2 diabetes. These trials include: first, hematopoietic stem cell transplantation in Type 1 diabetes patients at onset; second, autologous bone marrow-derived stem cell arterious infusion into the pancreas after femoral

catheterization in Type 1 and Type 2 diabetic patients; third, hematopoietic or mesenchymal stem cell and islet concomitant allotransplantation in Type 1 diabetic patients. Moreover, besides the possibility of regenerating the β-cell make-up, in recent years regenerative medicine has hypothesized the possibility of using stem cells and precursors to treat the complications of diabetes mellitus. For example, endothelial progenitor cells are currently being studied in clinical trials for treating neuropathy, cardiopathy and critical ischemia in the limbs of diabetic patients. Similar to endothelial progenitor cells, MSCs have also been used for the tissue repair of ulcers in the feet of diabetic patients.

Q Pluripotent stem cells, ES cells or adult stem cells: do any of these three show greater potential for treatment in diabetes?

■ **Piemonti**

I believe that the stem cell classification in adult, embryonic and fetal stem cells, amongst others, should be partially overcome. What is relevant, independent of the source, is the potency, and in my opinion the greater potential for treatment in diabetes lies in totipotent or pluripotent stem cells. This family includes ES cells, embryonic germ cells, reprogrammed somatic cells (by cell fusion, nuclear transfer or iPS cells), adult germline stem cells, amniotic fluid stem cells, endometrial regenerative cells, amniotic epithelial cells and very small embryonic-like stem cells. Out of these, iPS cells have the greater potential. They appear to satisfy virtually all criteria of true pluripotent cells (i.e., unique cellular morphology, gene expression profiles similar to that of ES cells, ability to form teratomas *in vivo* and the capacity to form germline transmissible chimeras upon injection into blastocysts). Compared with ES cells, the primary advantage of these cells is that they can be derived from the same individual for whom disease treatment is being sought. Thus, iPS cells are not subject to the same ethical issues that were raised for ES cell research, and moreover, their use may obviate the need for immunosuppression upon transplantation, at least in Type 2 diabetes. However, several limitations remain that raise some concerns for the use of iPS cells in a cell-based therapy. First, safety concerns persist. Aside from the worries over the use of viral vectors for cellular transduction, there are also concerns that the factors required for reprogramming may lead to tumorigenicity of derived cells upon transplantation. Moreover, even a small number of undifferentiated iPS cells present in the differentiated population could form teratomas in the transplanted tissues. Other limitations include the poor efficiency of iPS cell formation and the concern that the underlying genetic abnormalities that led to the disease in the first place may still be inherent in the derived cell types (particularly relevant for Type 2 diabetes).

■ **Domínguez-Bendala**

The jury is still out on the ability of adult stem cells to become *bona fide* β cells. This is especially true of MSCs, which are the workhorse of adult stem cell studies. This is because they appear to be committed along the mesodermal lineage, whereas the insulin-producing cells of the pancreas are endodermal. However, there is an increasing number of reports showing that some MSCs exhibit markers of more primitive cells, which opens the door to endodermal differentiation. It is worth noting that many adult stem cell therapies for diabetes (including those offered in some countries at for-profit clinics without any kind of regulation) are just based on the delivery of undifferentiated cells. Preliminary evidence in animal studies suggests that the effects of such interventions, if at all detectable, are likely to be indirect and due to the well-known immunomodulatory, proangiogenic and anti-inflammatory properties of MSCs.

As for ES versus iPS cells, the answer is less obvious than one would intuitively think. Is the pursuit of autologous therapies (including iPS cells) worth the trouble for Type 1 diabetes?

It could be argued that taking allorejection out of the picture would leave autoimmunity as the only remaining challenge, thus effectively halving the scope of the problem. All we would then need to do is to eliminate the autoimmune response. But is this not precisely the key to curing the disease? If we followed this logic, years and millions of dollars spent on the development of autologous β-cell transplantation strategies may just lead us to the realization that diabetes still needs to be cured. Therefore, the question of whether autologous is better than allogeneic in the context of Type 1 diabetes needs to be carefully pondered. The thrust of new technologies such as nuclear reprogramming – which are shaping the stem cell field in ways we could hardly imagine just a few years ago – may have contributed to the perception that some approaches must be explored just because they are within our reach. The decision

between allogeneic and autologous should be made before scarce resources are spread in too many directions. The idea of personalizing stem cell therapies for Type 1 diabetes may sound enthralling, but perhaps it will not get us any closer to a cure. A well characterized allogeneic stem cell substrate, in contrast, may be an easier solution to the problem – especially because until we cure autoimmunity or devise efficacious immunoisolation methods, immunosuppression will be needed, regardless of our choice.

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