Bernat Soria,[1,2] Eduard Montanya,[2,3,4] Franz Martín,[1,2] and Abdelkrim Hmadcha[1,2]

A Role for the Host in the Roadmap to Diabetes Stem Cell Therapy

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Stem cells represent an unlimited source for cell therapy (1), and considerable efforts have been made to overcome barriers to introducing this revolutionary therapy into clinical practice. Briefly, the following actions must be taken: *1*) design in vitro differentiation strategies to generate either mature postmitotic β-cells or β-cell progenitors that may be safely implanted into the host (e.g., without uncontrolled proliferation), *2*) devise selection methods to produce a pure β-cell population, *3*) validate standard characterization protocols to determine the real differentiation stage of the cells ready to be transplanted, *4*) obtain encapsulation devices to implant the cells, *5*) develop preclinical controls in representative animal models, and *6*) define cell-host interactions (for a recent review see ref. 2).

A breakthrough for in vitro differentiation and selection strategies was reported in *Diabetes* when it was shown for the first time that the process was doable (3). A combination of directed differentiation and gene-trapping strategies succeeded in manufacturing insulin-producing cells derived from rodent embryonic stem cells that normalized blood glucose when implanted into streptozotocin (STZ)-induced diabetic mice. Further reports improved the system by introducing new differentiation strategies, such as inhibiting sonic hedgehog or selecting cells expressing a gene also expressed in islet progenitor cells (Nkx6.1) (4). It was also shown that differentiated cells do not form teratomas (4,5) and they mature 30 days after transplantation (4). Subsequently, in vitro differentiation protocols for human embryonic stem cells (hESCs) or induced pluripotent stem cells succeeded in generating definitive endoderm (6–8). Numerous protocols have been published that have yielded insulin-producing cells from pluripotent stem cells (9–11). The full amount of contributions cannot be properly acknowledged in the limited space of this commentary. In vitro differentiation protocols reached a milestone in 2014 when a patient with diabetes received a subcutaneous implant of β-cell progenitors placed into a biocompatible capsule that could eventually be removed (Fig. 1) (12). This pilot study (clinical trial reg. no. NCT02239354, clinicaltrials.gov), the first one approved in patients with diabetes by the U.S. Food and Drug Administration (FDA), is currently recruiting participants with an estimated enrollment of 40 patients with type 1 diabetes. No information has been made public yet on the evolution of the implanted cells and patients.

In this issue, Bruin et al. (13) describe the impact of thyroid dysregulation on the in vivo maturation of β-cell progenitors derived from hESCs. Hypothyroidism was induced in SCID beige mice using an iodine-deficient diet with propylthiouracil. Hyperthyroidism was induced by the addition of T4 to drinking water. Thyroid dysfunction was described as "chronic" (for the duration of the study) or "acute" (4 weeks posttransplantation). Euthyroid, hypothyroid, and hyperthyroid mice received macroencapsulated, hESC-derived pancreatic progenitor cell transplants. Acute hyperthyroidism did not affect graft function, but hypothyroidism inhibited the maturation of transplanted islet progenitors and resulted in an increase in α- and ε-cells and fewer insulin-producing β-cells. Whereas acute hypothyroidism transiently impaired human C-peptide secretion, chronic hypothyroidism severely blunted human C-peptide secretion and glucose-stimulated insulin secretion and increased plasma glucagon levels. The results are in line with recent in vitro studies that have investigated the effects of thyroid hormones on the maturation of human β-cells (14). In hESCs differentiated toward β-cells, T3 enhanced the expression of the transcription factor MAFA and increased insulin content and insulin secretion in response to glucose (14). Bruin et al. (13) clearly show an altered maturation of pancreatic progenitors transplanted to hypothyroid mice. As grafts from chronic hypothyroid mice contained less β-cells and more α-cells, as well as reduced NKX2.2 expression, it may be

[1]CABIMER, Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain
[2]CIBERDEM, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders, Madrid, Spain
[3]Bellvitge Hospital-IDIBELL, Hospitalet de Llobregat, Barcelona, Spain
[4]University of Barcelona, Hospitalet de Llobregat, Barcelona, Spain

Corresponding author: Bernat Soria, bernat.soria@cabimer.es.

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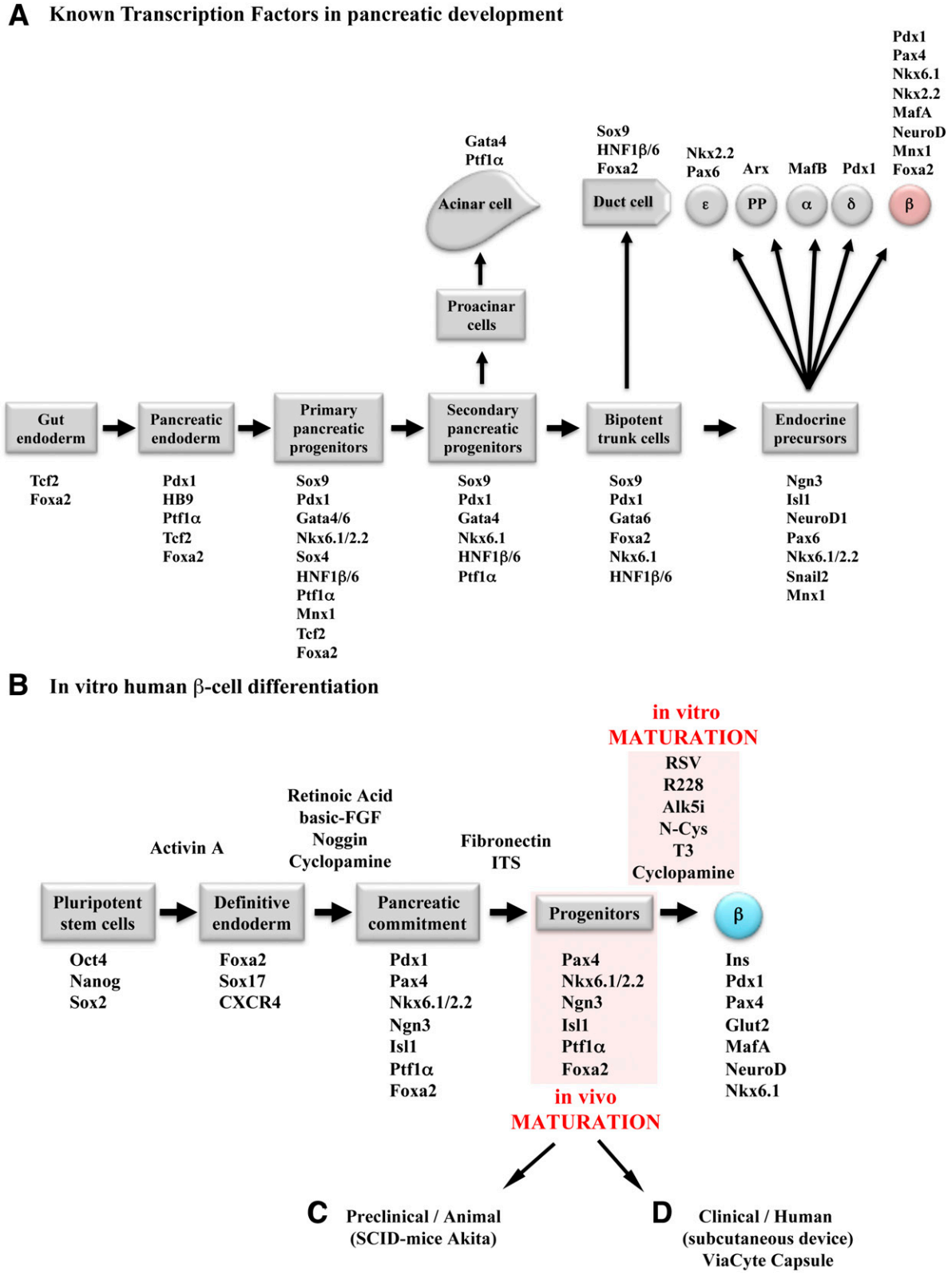

Figure 1—Simplified model of pancreas organogenesis and differentiation and maturation strategies to obtain a functional β-cell. *A*: Transcription factors required during the development of the islets of Langerhans. *B*: Sequential expression and temporal variation of genes during in vitro differentiation protocols. *C*: Pancreatic progenitors to specialize into functional β-cells through maturation after transplantation into STZ-induced hyperglycemic mice. *D*: Strategy for subcutaneous implantation of encapsulation devices with β-cell progenitors that can mature inside the patient's body and eventually control blood glucose and protect from immune insult. Alk5i, Alk5 receptor inhibitor; FGF, fibroblast growth factors; ITS, insulin transferrin selenium; N-Cys, N-acetyl cysteine; PP, pancreatic polypeptide producing cells; R288, small-molecule inhibitor of the tyrosine kinase receptor AXL; RSV, Resveratrol; T3, thyroid hormone T3.

that thyroid hormone deficiency could be tipping the balance in favor of a higher ARX expression that promotes the expansion of α-cells at the expense of β- and δ-cells. Bruin et al. (13) recommend the transplantation of more mature hESC-derived cells to minimize the maturation period after transplantation. We suggest that transplantation of MAFA-positive, hESC-derived cells could overcome the effects of chronic hypothyroidism. However, at this point the concept that reduced levels of thyroid hormones may drive the differentiation of pancreatic progenitors cells toward α- and ε-cell lineages at the expense of β-cell formation, although attractive, requires further study. On the other hand, a key observation is that these progenitors may generate different islet cell types resulting in a cell aggregate that could potentially better simulate islet behavior.

The take-home message of the study by Bruin et al. is that host milieu is determinant in the maturation process of hESC-derived progenitor cells toward β-cells. Thus, recipient candidates for this therapy may require additional screening to fulfill the inclusion criteria. Relevant issues that must be addressed in the field of stem cell therapy in diabetes are *1*) preimplantation maturation, and β-cell selection strategies, *2*) degree of maturation of differentiated cells, *3*) patient inclusion criteria, *4*) safety of implantation devices, *5*) lack of long-term data, *6*) role of non–β-cells (α-cells, ε-cells, etc.) in the islet physiology, *7*) vascularization of pseudoislet structures and the role of endothelial cells, *8*) development of fibrotic tissue around the capsule restricting the access of oxygen and metabolites, and *9*) number of cells that should be implanted.

Regulatory authorities such as the FDA or the European Medicines Agency (EMA) have established that cells used to treat diseases are "cellular medicaments" that must fulfill the same criteria as small molecules and biologicals (15). So far the only established cell therapy is bone marrow transplantation. Cell therapy for diabetes is still an experimental cellular medicament and has to follow the well-established pattern of Phase I (safety), II (efficacy and safety), and III (efficacy, efficiency, and safety) trials to be approved by the regulatory agencies. Our view is that there is a need to improve these cellular medicaments by iterative preclinical and clinical research. Because of the high cost of this development program, we wonder whether these medicaments will be cost effective and affordable for the millions of people that suffer from diabetes. In this sense, and given the enormous task that the field faces, a private–public international consortium with transparent rules may be a more efficient way to reach a safe, effective, and affordable cure for type 1 diabetes.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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