Type 1 diabetes mellitus is one medical condition likely to benefit from stem-cell therapy: insulin-secreting pancreatic beta cells derived from stem cells — pending availability — could be transplanted into patients and help maintain blood glucose homeostasis. The relative success of the transplantation of cadaveric beta cells into patients with type 1 diabetes\(^1\) provides support for this approach. The theoretically unlimited supply of beta cells derived from stem cells could translate into a better life for countless patients. Although there has been much discussion about the particulars of such transplantations, including the optimal delivery site, the composition of the transplanted tissue, and the role of immunosuppressive drugs, there has been surprisingly little discussion about how beta cells will be derived from stem cells in the first place.

Studies in the past few years have indicated that we need to recreate in the cell culture dish what happens in the embryo. Thus, a stem cell must first be induced to make definitive endoderm, then differentiate into pancreatic (or \(PDX1\)-expressing) endoderm, and finally reach a stage in which it secretes insulin in response to high glucose concentrations. The molecular details of this differentiation pathway are under intense scrutiny and yet remain sufficiently obscure to prevent a clear understanding of the challenges at hand. It comes as welcome news, therefore, that D'Amour et al.\(^2\) have coaxed human embryonic stem cells into the endodermal lineage in culture.

Because studies in frogs, zebrafish, and mice have clearly documented the critical role of transforming growth factor \(\beta\) (TGF-\(\beta\)) signaling in the formation of endoderm,\(^3\) D'Amour et al. bathed human embryonic stem cells in culture medium that included activin, a member of the TGF-\(\beta\) family (Fig. 1). They also lowered the concentration of serum in the culture medium to minimize signaling that might interfere with activin-induced signaling. They thereby produced cultures consisting of up to 80 percent endodermal cells, as reflected by the expression of several key markers and by morphologic features. They assessed the ability of this enriched cell population to differentiate by transplanting it beneath the kidney capsule in mice, whereupon it produced intestinal and hepatic-like tissues. Most important, the authors identified a chemokine receptor on the surface of endodermal cells (Fig. 1) — a receptor that is also expressed on endodermal cells of mice and zebrafish. The presence of this marker allowed them to sort the cultured cells, leading to a population of nearly 100 percent endoderm.

In addition to providing useful material for further differentiation into medically beneficial types of cells, such as pancreatic beta cells and hepatocytes, the work of D'Amour et al. also improves our understanding of the differentiation process that results in human endoderm. Cultures of cells differentiating relatively synchronously into the endodermal lineage could be studied to decipher the underlying transcriptomes (the complete RNA complement of a cell) and proteomes (the complete protein complement of a cell) at various steps of the process. Furthermore, the application of this protocol to the more easily manipulated mouse embryonic stem cells should open up productive avenues of research, including further investigation of the genetic requirements for this differentiation process.

The key to stem-cell therapy is the ability to generate large numbers of differentiated cells. (For example, the Edmonton protocol, which has been used successfully for the transplantation of human pancreatic islets, requires approximately \(2\times10^8\) to \(7\times10^8\) beta cells per patient.) Although
insulin-producing beta cells can divide,\(^5\) it will probably be easier to increase the number of cells earlier in the process of differentiation. However, we know little about the driving force behind the proliferation of progenitor cells in the embryo, and because of the propensity of the embryo to regulate the number of cells in each lineage, it may be easier to decipher this process with the use of tissue culture.

How far are we from being able to generate pancreatic beta cells from stem cells? One way to evaluate this question is to consider the time frame of this differentiation process in a fast-developing vertebrate embryo. In zebrafish, endodermal cells, which express \textit{sox17}, appear approximately 5 hours after fertilization, and pancreatic endodermal cells, which express \textit{pdx1}, are first seen 14 hours after fertilization. Multiple signaling and consequent gene-expression events can certainly take place during the intervening nine hours, and we are just beginning to learn about them. It is, in fact, likely that the transition from the expression of \textit{sox17} to that of \textit{pdx1} represents multiple steps and that these steps will first need to be identified, either in the embryo or in the tissue-culture dish, before further progress can be made.

Undoubtedly, the road to stem-cell therapy for type 1 diabetes will be long and bumpy. The protocol of D’Amour et al.\(^2\) will, of course, need to be validated in other laboratories before being
widely used. However, the rigor of their study sets a good standard for the work yet to come.

Dr. Stainier reports serving as a consultant for Cythera. No other potential conflict of interest relevant to this article was reported.

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