Stem cells and a cure for type 1 diabetes?

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The discovery that adult stem cells can be reprogrammed, backwards, to induced pluripotent stem cells (iPS) was a remarkable and landmark breakthrough in 2006 (1). These iPS then can be differentiated by using specific gene transfections into a wide variety of cell types. Now, at a tremendous pace, many laboratories are improving the efficiency and homogeneity of this process, including the replacement of gene transfection with proteins and small molecules (2). Perhaps not surprisingly, it has been shown that iPS can be made from adult cells from people with diseases (e.g. ref. 3), and in this issue of PNAS Maehr et al. (4) illustrate this process for type 1 diabetes. Type 1 diabetes is one of the most common diseases in childhood, causing significant morbidity and mortality and enormous healthcare and economic costs. Worse still, its incidence in children aged under 5 years is set to double by 2020 (5). Currently, we have no idea how to prevent this rapid increase, which must be caused by an increasing permissiveness of the environment acting on a genetic susceptibility background in many countries worldwide.

What are the implications of this advance for research and clinical application in type 1 diabetes? As Maehr et al. (4) state, the clinical applications are a very long way off. Safety is paramount, and cellular therapies will require rigorous clinical evaluations, especially given the possibility that transplanted cells could conceivably change their phenotype and functions in vivo and have harmful effects. Such alterations in phenotype or effect could depend on a patient's genotype or exposure to environmental factors, such as infection. In type 1 diabetes, the specific challenge is that any transplant of pancreas, purified islets, or engineered insulin-expressing, glucose-responsive cells will be rapidly destroyed by the body's own immune system, in the absence of immunosuppression or (and this is an active, major research activity) in the absence of induced antigen-specific tolerance, which could be safer than any form of general immunosuppression (6). Type 1 diabetes results from an inherited loss of immune tolerance to insulin and its precursors and other pancreatic islet antigens, leading to destruction of the insulin-producing islet β cells by the autoimmune activities of antigen-presenting cells such as B lymphocytes, macrophages, dendritic cells, and CD4 and $CD8^+$ T lymphocytes. The anti-islet memory T cell response, once established, is very strong and long-lasting, akin to the lifelong protection provided by memory T cells against infections.

Therefore, the exciting and nearer-term implications of type 1 diabetes-specific iPS (DiPS) cells is in research.

One goal is to make DiPS that are resistant to autoimmune attack.

If the differentiation process into insulin-producing, glucose-responsive cells can be made much more efficient, perhaps combined with cell sorting or enrichment, then researchers can use these cells in in vitro studies to study interactions with the patients' own immune cells from peripheral blood samples or immune cells produced by iPS technology. These DiPS could come from donors with genotypes associated with type 1 diabetes; for example, there is evidence that the immune etiology of type 1 diabetes is different in cases of HLA-DR3/4 heterozygotes versus DR3/3 or DR4/4 homozygotes. Clearly, the DR3/4 genotype has been associated with a decreased age at diagnosis and perhaps interacts differently with the unknown, but increasingly permissive, environmental factors (7). These HLA class II genes are the major genetic effect in type 1 diabetes (8), and the frequencies of the various susceptibility alleles and haplotypes correlate with the incidence of type 1 diabetes across several countries. One goal is to make DiPS that are resistant to autoimmune attack. In the widely used spontaneous mouse model of autoimmune type 1 diabetes, the nonobese diabetic (NOD) strain, >10 genes have been tested over the past 10 years in transgenic modification of NOD mice to try to make their β cells resistant to autoimmune destruction in vivo. The most successful example, with the least side effects, was the β cell expression of the decoy receptor 3 gene (DCR3 or TR6) (9).

It may be possible to examine the interactions of DiPS immune cells and β cells in vivo in humanized mice by transfer, for example, into an immune-deficient version of the NOD strain in which the immune genes *scid* and *il2rg* have been knocked out (10). However, this approach may be limited by the extent that the mouse model can be humanized and remain physiologically relevant to the human disease. Furthermore, we understand relatively little about the normal and disease-affected immune systems of humans compared with the mouse (11, 12). Meanwhile, the spontaneous NOD model remains an invaluable experimental tool and preclinical model, not least because it has genetic alterations in pathways that are directly conserved with human genetic susceptibilities, including the HLA or MHC class II molecules, the IL-2 pathway, and T cell activation pathways (13, 14). This conservation is remarkable and the extent of it will likely increase as we continue to map and identify the genes that affect the human disease. Nevertheless, it is probably not that surprising if we consider that many of these rate-limiting functions in the immune system, manifested by the existence of common functional polymorphisms in mouse, rat, and human populations, have been under Darwinian selection in mammals in the constant and ancient war against pathogens. Some authors have criticized the NOD model in that it has not led to successful human clinical trials (15), but I suggest the real reasons for the current failures of several prevention trials of type 1 diabetes lie in our remaining state of ignorance (about disease etiology) and consequent inadequate design of the trials (the wrong dose of reagent, wrong timing, wrong delivery, too little, too late, and the necessity of safety first especially in children) (6, 16). No one will accept any risks of altering the immune response of a child in a way

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that could increase the risk of cancer (www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm175803.htm) or cause a defect in immune defense to a dangerous pathogen (17). These considerations are a driver for identifying the many common environmental exposures that modify the genetic risk of the disease and account for the steady rise of type 1 diabetes in children, its north–south gradient and striking seasonality.

We need integrated and collaborative mouse and human research programs in the study of immune systems using the latest technologies and well-defined and quality-controlled resources that can be shared and cross-validated. iPS technology and other recent successes will be an exciting part of this future.

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