to be developed. Cells tend to develop abnormal karyotypes and other abnormalities with increased time in culture, and ES cells have the potential to form teratomas if all cells are not committed to the desired cell types before transplantation. Further, human ES cells are ethically controversial and, on these grounds, would be unacceptable to some patients and physicians despite their therapeutic potential. Nevertheless, there have been limited clinical trials of ES-derived cells in a number of disorders, including macular degeneration, myopia, and spinal cord injury.

Induced Pluripotent Stem Cells The field of stem cell biology was transformed by the discovery that adult somatic cells can be converted ("reprogrammed") into pluripotent cells through the overexpression of four transcription factors normally expressed in pluripotent cells **(Chap. 88)**. These iPS cells share most properties with ES cells, although there are distinct differences in gene expression between ES and iPS cells. The initial use of viruses to insert the transcription factors into somatic cells made the resulting cells unsuitable for clinical use. However, a number of strategies have since been developed to circumvent this problem, including the insertion of modified mRNAs, proteins, or microRNAs rather than cDNAs; the use of non-integrating viruses such as Sendai virus; the insertion of transposons with the programming factors, followed by their subsequent removal; and the use of floxed viral constructs, followed by treatment with Cre recombinase to excise those constructs. The safety of iPS cells in humans remains to be demonstrated, but clinical trials in macular degeneration and other disorders are planned. Potential advantages of iPS cells are that somatic cells from patients would generate pluripotent cells genetically identical to those of the patient and that these cells are not subject to the same ethical constraints as ES cells. It is not clear whether the differences in gene expression between ES and iPS cells will have any impact on their potential clinical utility, and studies of both cell types will be essential to resolve this issue.

Umbilical-Cord Stem Cells Umbilical-cord blood stem/progenitor cells (USCs) are widely and readily available. These cells appear to be associated with less graft-versus-host disease than are some other cell types, such as marrow stem cells. They have less human leukocyte antigen restriction than adult marrow stem cells and are less likely to be contaminated with herpesvirus. However, it is unclear how many different cell types can be generated from USCs, and methods for differentiating these cells into nonhematopoietic phenotypes are largely lacking. Nevertheless, there are ongoing clinical trials of these cells in dozens of disorders, including cirrhosis, cardiopathies, multiple sclerosis, burns, stroke, autism, and critical limb ischemia.

Organ-Specific Multipotent Stem Cells Organ-specific multipotent stem cells have the advantage of already being somewhat specialized so that the inducement of desired cell types may be easier. Cells potentially could be obtained from the patient and amplified in cell culture, circumventing the problems associated with immune rejection. Stem cells are relatively easy to harvest from some tissues, such as bone marrow and blood, but are difficult to harvest from other tissues, such as heart and brain. Moreover, these populations of cells are more limited in potentiality than are pluripotent ES or iPS cells, and they may be difficult to obtain in large quantities from many organs. Therefore, substantial efforts have been devoted to developing techniques for using more easily obtainable stem cell populations, such as bone marrow mesenchymal stem cells (MSCs), CD34+ hematopoietic stem cells (HSCs), cardiac mesenchymal cells, and adipose-derived stem cells (ASCs), for use in regenerative strategies. Tissue culture evidence suggests that these stem cell populations may be able to generate differentiated cell types unrelated to their organ source (including myocytes, chondrocytes, tendon cells, osteoblasts, cardiomyocytes, adipocytes, hepatocytes, and neurons) in a process known as *transdifferentiation*. However, it is still unclear whether these stem cells are capable of generating differentiated cell types that integrate into organs, survive, and function after transplantation in vivo. A number of early studies of MSCs transplanted into heart, liver, and other organs suggested that the cells had differentiated into organ-specific cell types with beneficial effects in animal models of disease. Unfortunately, subsequent studies revealed that the stem cells had simply fused with cells resident in the organs and that the observed beneficial effects were due to paracrine release of trophic and anti-inflammatory cytokines. Further studies will be necessary to determine whether transdifferentiation of MSCs, ASCs, or other stem cell populations occurs at a high enough frequency to make these cells useful for stem cell replacement therapy. Despite the remaining issues, clinical trials of MSCs, autologous HSCs, USCs, and ASCs are being performed in many disorders, including ischemic cardiac disease, cardiomyopathy, diabetes, stroke, cirrhosis, and muscular dystrophy.

Regardless of the source of the stem cells used in regenerative strategies, a number of generic problems must be overcome for the development of successful clinical applications. These problems include the devising of methods to reliably generate large numbers of specific cell types, to minimize the risk of tumor formation or proliferation of inappropriate cell types, to ensure the viability and function of the engrafted cells, to overcome immune rejection when autografts are not used, and to facilitate revascularization of regenerated tissue. Each organ system will also pose tissue-specific problems for stem cell therapies.

DISEASE-SPECIFIC APPLICATIONS OF STEM CELLS

Ischemic Heart Disease and Cardiomyocyte Regeneration Because of the high prevalence of ischemic heart disease, extensive efforts have been devoted to the development of strategies for stem cell replacement of cardiomyocytes. Historically, the adult heart has been viewed as a terminally differentiated organ without the capacity for regeneration. However, recent studies have demonstrated that the heart has the capacity for low levels of cardiomyocyte regeneration **(Chap. 265e)**. This regeneration appears to be accomplished by cardiac stem cells resident in the heart and possibly also by cells originating in the bone marrow. The heart might be an ideal source of stem cells for therapeutic use, but techniques for isolating, characterizing, and amplifying large numbers of these cells have not yet been perfected. For successful myocardial repair, stem cell therapy must deliver cells either systemically or locally, and the cells must survive, engraft, and differentiate into functional cardiomyocytes that couple mechanically and electrically with the recipient myocardium. The optimal method for cell delivery is not clear, and various experimental and clinical studies have successfully employed intramyocardial, transendocardial, intravenous, intracoronary, and retrograde coronary venous injections. In experimental myocardial infarction, functional improvements have been achieved after transplantation of a variety of different cell types, including ES cells, HSCs, MSCs, USCs, and ASCs. Early studies suggested that each of these cell types might have the potential to engraft and generate cardiomyocytes. However, most investigators have found that the generation of new cardiomyocytes by these cells is at best a rare event and that graft survival over long periods is poor. The preponderance of evidence suggests that the observed beneficial effects of most experimental therapies were not derived from direct stem cell generation of cardiomyocytes but rather from indirect effects of the stem cells on resident cells. It is not clear whether these effects reflect the release of soluble trophic factors, the induction of angiogenesis, the release of anti-inflammatory cytokines, or another mechanism. A wide variety of cell delivery methods, cell types, and cell doses have been used in a progressively enlarging series of clinical trials, but the fate of the cells and the mechanisms by which they alter cardiac function are still open questions. In aggregate, however, these studies have shown a small but measurable improvement in cardiac function and, in some cases, reduction in infarct size. In short, the available evidence suggests that the beneficial clinical impact reflects an indirect effect of the transplanted cells rather than genuine cell replacement.

Diabetes Successes with islet cell and pancreas transplantation have provided proof of concept for cell-based therapies for type 1 diabetes. However, the demand for donor pancreases far exceeds the number available, and maintenance of long-term graft survival is a problem. The search for a renewable source of stem cells capable of regenerating pancreatic islets has therefore been intensive. Pancreatic beta cell