Cellular Transplants

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THE TERM "cell" was coined in 1665 by Hooke[1] who described "little boxes or cells distinct from one another" while examining a piece of cork with a microscope. The first cell transplant in humans may have been a blood transfusion performed in 1492. A story of ambiguous authenticity describes Pope Innocent VIII as the first recipient of a cell transplant, the blood transfusion donors being three youths. The donors and recipient passed on shortly thereafter, and the prescribing transplant physician disappeared in an unknown direction.[2]

The world of cellular transplantation was pioneered by the early work in tissue culture and transplantation by Zahn (1878), Arnold (1887),[3] Williams (1893),[4] Born (1896), Harrison (1905),[3] and Carrel (1910).[5] A milestone in cell transplant history was the introduction of proteolytic enzymes to dissociate tissues into single cells by Rous and Jones in 1916 who used trypsin to separate growing cells from tissue included in a plasma clot.[6] Tissue had been grown by the technique of Carrel and Burrow as modified from Harrison's method.[5] The liberation of the new cells by trypsin digestion was not widely accepted because of the fear of damaging the cells by the enzymatic treatment.[7] Thirty years later, trypisinization and the isolation of collagenase from *Clostridium* welchii in 1946[8] opened the way to modern technology of cell separation and transplantation (Table 1).

Several cellular-transplant models have been described (Table 2), and many of them share problems and advantages, as can be exemplified with work on pancreatic islets. Separation and/or purification of an adequate number of intact islets is the first requirement.[9] The second main problem has been the lack of effective procedures for early detection and treatment of rejection episodes. Because of the small cell mass, it is too late to treat a rejection episode when it becomes manifest by functional failure of the graft. This problem is particularly difficult when immature or fetal cells are transplanted. Here, a significant gap exists beween the time of transplantation and the beginning of functional activity of the grafted cells. The fact that the transplant has been destroyed during this silent period is realized only when it does not function later.

Table 1. Early Development In Enzymatic Cell Separation Methods

1916	Rous and Jones	Cell separation from plasma clot by proteolytic digestion (trypsin)
1946	Oakley	Isolation of collagenase from *C. welchii*
1956	Rappaport	Automatic method for the preparation of cell suspension (trypsinization of monkey kidney tissue)
1958	Rinaldini	Purified collagenase; isolation of living cells from animal tissues

Despite these challenges, cellular transplantation offers several advantages compared with organ transplantation, of which the first is the ease of the surgical procedure which, in many cases, is a simple injection. In addition, it is possible to manipulate tissue in vitro before transplantation to inactivate and/or destroy class II positive antigen-presenting cells[9-13] (Table 3).

Also, it may be feasible with encapsulation techiques to erect a physical barrier between the transplanted cells and the recipient's immune systems.[14-16] The application of the microencapsulation technology is currently limited by the need for materials that do not stimulate fibroblastic response in the recipient. Macroencapsulation intravascular devices require nontrombogenic surfaces that are resistant to fibrin deposition while they maintain an effective interface with blood and/or tissue fluids.

Another potential advantage of cellular transplant is the possibility of cryopreserving the cells.[17,18] This would allow the creation of banks of tissue for use at a later time. This will be crucial where multiple donors are needed for an adequate number of cells. Besides answering quantitative needs, transplantation from multiple donors has resulted in induction of tolerance in experimental models.[19-20]

Appropriate sites for cellular transplantation are necessary to ensure the adequate vascular support that is essential for integration and reconstitution of any biologic function. The site choice also may be immunologically relevant, and may influence the intensity of allograft and xenograft rejection and survival.[21-25]

Recently, Thompson et al reported the formation of neovascular structures after implantation of fibers coated with collagen, and growth factors in the peritoneal cavity of rats.[26] These organoids have been capable of sustaining the biologic function of implanted hepatocytes. Besides providing a favorable environment for engraftment and function, organoids could be an alternative to diffuse

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Table 2. Types of Cellular Transplants

Adrenal
Bone marrow
Epidermal
Endothelial
Fibroblasts
Hepatocytes
Islets
Lymphocytes
Myoblasts
Neural
Parathyroid
Stem cells

intraperitoneal implantation of cells, making it possible to confine the cellular implant to a well-defined, vascularized space that could be easily removed in case of adverse reaction. This approach[26] could be applied for the implantation of autologous cells after restoration of a deficient function by gene transfer.

Gene therapy is, in fact, a powerful first cousin of cellular transplantation that greatly amplified its potential applications. With gene therapy, viruses or other transfecting agents, stripped of their deleterious functions, can be used as vehicles to introduce normal, functional genes into cultural human cells with deficient genomes and, thus, to cure genetic diseases. Recent reviews on the subject are available.[27-30]

Apart from gene therapy, the feasibility of somatic correction of inborn errors with cells has been demonstrated beyond question by bone marrow transplantation of consanquineous and closely-matched allografts.[29] Consequently, bone marrow technology has been a magnet for studies of gene therapy. The more widely-studied possibilities have been the immunodeficiency diseases caused by defects of adenosine deaminase and purine-nucleoside phosphorylase, chronic granulomatous diseases, and Gaucher's disease. Other diseases, such as erythroid cell disorders of hemoglobin expression, including sickle cell anemia and the thalassemias, could become also theoretical targets for gene therapy if appropriate regulation and efficient expression of globin genes can be introduced into the deficient, isologous bone marrow of the patients. This would provide the benefits of bone marrow allografts without the penalties of rejection and/or graft-versus-host disease.

Because of its determinant role in the expression of many genetic and metabolic diseases,[31] the liver is another target for gene therapy.[32] Several genes have been expressed in primary hepatocyte cultures in vitro, including the disease-related genes for the human receptor for low-density liproteins, phenylalanine hydroxylase, and alpha-1-antitrypsin. Research efforts are now concentrated to develop efficient methods for the implantation of genetically-modified hepatocytes, and/or to develop vectors that can be introduced directly into hepatocytes in vivo. Correction might then become possible without in vitro genetic manipulation and cell reimplantation.

Several models of gene therapy have been proposed for central nervous system genetic and nongenetic diseases.[27] The genetic approaches to therapy of central nervous system disorders are of difficult application because most target cells are postmitotic (neurons) and, therefore, refractory to infection with retroviral vectors. In addition, most disorders affecting the central nervous system function are probably multigenic and multifactorial, and the target cells are located in sites that are not easily accessible. Neurotrophic DNA viruses, such as herpes simplex virus, however, have exciting new potential for gene delivery to the central nervous system. In this case, apathogenic strains that establish life-long latent infections of brain cells while expressing only nonviral therapeutic genes from the latent viral genomes are under development (J.C. Glorioso, personal communication, August 1990). Large DNA viruses have the advantage that multiple minigene cassettes can be introduced in a site-specific manner and will be stably expressed without direct integration into neural cell chromosomes.

Studies are now underway in many centers to examine the potential role of retrovirally transduced mouse nerve growth factor cDNA for the treatment of Alzheimer's disease. Similar approaches have been studied for the delivery of useful agents in models of Parkinson's disease.

Gene therapy also has been proposed for cancer treatment.[27] Deficiencies of cancer-suppressor genes, such as those apparently associated with retinoblastoma and Wilms' tumor, could be treated by restoration of the suppressor-gene expression. Other approaches involve inactivation of dominantly-acting oncogenes.

Gene transfer could be used to endow cell transplants with new functions that they do not normally possess. For example, cultured fibroblast isografts could be reintroduced after the introduction of genes to replace defective hormones such as insulin,[33] serum proteins, and other metabolic products.

Another potential advantage of cellular transplantation is the possibility to use combined cellular transplants. In this case, a cell population is transplanted to allow or improve the survival of a second-cell type, as in the case of pancreatic islets that promote the survival of hepatocytes transplanted in ectopic sites.[34] Moreover, adrenal cortical cells may provide local steroid secretion[35] to protect other cell types from rejection (unpublished observations).

Time constraints make it impossible to review all cell-

Table 3. Immunoalteration Techniques

Culture (95% O_2, 24°C, hyperbaric, hyperthermic)
Cryopreservation
Antibodies (anti-class II Ag, -dendritic cells, -IL)
Ultraviolet light, donor or tissue, irradiation
Single cell dispersion and reaggregation
Donor fatty acid deficiency
Anti-oxygen–free radical reagents

transplant applications. Pancreatic islet transplantation can be chosen to show that significant clinical progress is being made in the field. The procedures for isolation of the islet cells from exocrine tissue were pioneered by Moskalewski[36] and Lacy.[37] In 1967, Lacy introduced the concept of pancreatic distension and collagenase digestion for islet preparation[37] which remains the basis of isolation technology today.[9,38-44] The most recent data of the islet allograft registry by Hering et al indicate that, before 1984, no islet transplant demonstrated significant islet function (basal C-peptide >1 ng/mL) 1 month after transplantation.[44] Twenty-six adult islet allografts were performed between 1985 and 1989, and, in over 30% of these cases, basal C-peptide production was observed in the first week posttransplant. However, less than 20% had documented C-peptide production 1 month after transplantation. 1990 has been a critical year for islet transplantation. It was recently reported by the Washington University group that allogenic purified human islets transplanted intraportally had resulted in exogenous insulin independence for 2 weeks in a patient with type I diabetes.[45] At the University of Pittsburgh, (starting January 10, 1990) we performed a series of human islet allografts in nine patients who were diabetic as a result of an upper abdominal exenteration which included radical pancreatectomy that was performed for extensive malignancies.[46] These patients received islet-cell allografts at the time of or just after the liver replacement.[47] They had monotherapy with FK 506 for immunosuppression. As of August 15, 1990, six of the nine patients are alive, 132 to 217 days postoperatively. Five are insulin-free or are on insulin only during periodic parenteral alimentation. The first islet transplant of this series was performed in a 15-year-old girl who requires neither parenteral alimentation nor insulin. She received islets from a single donor (same as the liver) and, to our knowledge, is the first unequivocal example of a successful islet-cell transplant in humans. It is noteworthy that both the donor and recipient were children, and also, that only a single islet donor was used.

Significant C-peptide production was documented in all nine of the transplanted patients. Subsequently, three other centers (St Louis, Milan, Edmonton) have achieved prolonged insulin independence after islet allotransplantation in type I diabetic patients (P.E. Lacy, C. Socci, R.V. Rajotte, personal communication, August 1990). This burst of results from four geographically-separated institutions will be a stimulus for further clinical applications. The size and quality of the yield of islet cell mass has been improved, but other factors remain to be clarified, such as the adequacy of various implantation sites for islet engraftment and revascularization; the diagnosis and treatment of rejection; and the diabetogenic affect of the immunosuppressive agents, including FK 506, cyclosporine, and, above all, steroids.

Islet transplantation is an example of a cell transplant procedure that finally has reached the prospect of success in clinical trials this year after several decades of intensive research. The unselfish exchange of information between centers and scientists have been fundamental to progress.

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