CELLULAR TRANSPLANTATION AND GENE THERAPY

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The first recipient of a cell transplant (blood transfusion) could have been Pope Innocent VIII in 1492, the donors being three youths. Donors and recipient died and no record of the procedure was left by the prescribing physician who disappeared under mysterious circumstances (1).

Almost two centuries later the term "cell" was coined by Hooke (1) who described "little boxes or cells distinct from one another" in a piece of cork examined at the microscope.

Pioneers in the early work on tissue culture and transplantation were Zahn (1878), Arnold (1887)(3), Williams (1893) (4), Born (1896), Harrison (1905) (3) and Carrel (1910) (5), but the real milestone in cell transplant history has been the introduction of protealitic enzymatic digestion to dissociate cells from tissue and organs. Rous and Jones first introduced trypsin to separate growing cells from tissue included in a plasma clot (6), but fear of damaging the cells by the enzymatic treatment (7) delayed the diffusion of the technique. The subsequent isolation of collagenase from Clostridium welchii in 1946 (8) opened the way to the modern technology of cell separation and transplantation.

Cellular transplant models share problems, advantages, and research targets.

SEPARATION/PURIFICATION

Retrieval of an adequate number of morphologically and functionally intact cells is the first requirement. Enzymatic digestion of tissues can potentially damage the cells during the separation procedure (7). Therefore, the progress in cell

separation technology has paralleled improvement in experimental and clinical transplantation results (9).

REJECTION

The major problem of cellular transplantation is the lack of effective procedures for early detection and treatment of rejection. The relatively small cell mass that is transplanted generally leads to difficulty in early diagnosis of rejection. In most of the cases it is too late to treat a rejection episode when it becomes manifest. The problem is even more dramatic when immature or fetal cells are transplanted because of the gap between the time of implantation and the beginning of functional activity of the graft that can be of months before any significant functional activity is detected. The absence of early graft function makes it even more difficult to detect and treat a rejection episode.

Despite these problems, cellular transplantation offers unique advantages compared to vascularized organ grafts. EASE OF IMPLANTATION

In many cases a cell transplant is a simple injection or cell infusion that can be performed under local anesthesia. Percutaneous and laparoscopic approaches are also currently under evaluation.

PRE-TRANSPLANT CELL TREATMENT

The prospect to avoid continuous recipient immunosuppression by in-vitro treatment of the tissue before transplantation to decrease the immunogenicity of the graft is attractive (9-14). Several treatments have been proposed in the last 15 years,

including low temperature, high oxygen, hyperthermic and hyperbaric cultures, monoclonal and policlonal antibody treatment, radiation, single cell dispersion and sorting and cryopreservation.

IMMUNOISOLATION TECHNIQUES

The introduction of a physical barrier between the transplanted cells and the recipients immune system (15-17) is an attractive possibility and significant progress has been made towards the development of materials that do not stimulate fibroblastic response in the recipient. Clinical trials using immunoisolation devices are in progress and will provide critical information in the near future.

CRYOPRESERVATION AND BANKING

The possibility to cryopreserve cells (18-19) makes it possible to create banks of tissue for transplantation. This technology will allow to delay the time of transplantation, for example to provide sufficient time to induce donor specific unresponsiveness or tolerance. In addition, multiple donors could be used to increase the number of cells available to the recipient. Transplantation from multiple donors has been reported to result in graft acceptance in experimental models (20-21). Cryopreservation have been recently proposed also as a mean of immunoalteration of the tissue before transplantation.

SITES OF IMPLANTATION

Appropriate sites of implantation are required to ensure adequate vascular support for integration and reconstitution of the cellular graft. Transplantation in different sites can

result in different allograft and xenograft survival (22-26)
introducing a̶n̶d̶ *a* immunologically relevant variable. In addition,
the microenvironment at the transplant site can affect
engraftment of the transplanted cells. For example, the exposure
to cytokines and the intrinsic ability of tissues to generate
nitric oxide could lead to impaired engraftment/function of
transplanted cells.

The confirmation of the recent report of the formation of
organoid neovascular structures after implantation of fibers
coated with collagen and growth factors in the peritoneal cavity
of rats (27) could lead to a variety of cellular transplant
applications including implantation of autologous cells after
restoration of a deficient function by gene transfer. In
addition, the organoid neovascular structure makes it possible to
confine a cellular implant in a well-defined, vascularized space
which could be easily removed in case of adverse reaction.

GENE THERAPY

Viruses or other similar agents can be used as vehicles to
introduce functional genes into human cells. Many cellular
defects and genetic diseases could be treated by this approach
(28-31). Approaches for gene therapy include gene replacement,
gene correction, and gene augmentation. A mutant gene sequence
can be replaced with a normal functional gene (gene replacement).
More ideally, specific correction of a mutant gene sequence could
be performed without any additional change in the target genome
(gene correction). As an alternative, gene augmentation could be

used to modify the expression of the content of a mutant gene in defective cells. Several methods are available to deliver genes into mammalian cells (28) including: 1) co-precipitation with calcium phosphate; 2) use of polycations or lipids to complex with the DNA; 3) encapsidation of DNA into liposomes or erythrocyte ghosts; 4) exposure of the target cells to rapid pulses of high voltage current and 5) introduction of DNA into cells by direct microinjection.

Retroviruses have been the most used viral vectors for their ability to infect a broad class of cell types. Nevertheless, they require cell replication and DNA synthesis, restricting their efficient use to cells that are able to replicate. Their characteristic of random integration in the cell genome introduces an additional negative factor: the risk of insertional mutagenesis. Other vectors such as adeno-associated virus (AAV) and herpesviruses have been developed. In particular, AAV viruses are ubiquitous in humans and can be concentrated to very high titles. They are not pathogenic and require helper adenovirus or herpesvirus for replication. In addition, it is possible to achieve constant integration site decreasing the risk of mutagenesis that derives from random integration.

Bone marrow has been one of the most attractive targets for gene therapy (30), the more studied models being the immunodeficiency diseases caused by defects of adenosine deaminase (ADA) and purine-nucleoside phosphorlyase, chronic granulomatous diseases and Gaucher's disease. Erythroid cell disorders of hemoglobin expression, including sickle cell anemia

and the thalassemias are also theoretical targets for this approach.

Another target for gene therapy is the liver (33) and several genes have been expressed in primary hepatocyte cultures, including the disease-related genes for the human receptor for low-density liproteins, phenylalanine hydroxylase, and alpha1-antitrypsin. Research is now focusing on methods for the implantation of genetically modified hepatocytes and on the development of vectors that can be introduced directly into hepatocytes in-vivo.

Models of gene therapy have been proposed for central nervous system diseases (28). Challenges in these applications include the fact that most target cells are postmitotic (neurons) and therefore refractory to infection with retroviral vectors. In addition, several disorders affecting the central nervous system are likely to be multigenic and multifactorial, the target cells being located in sites that are not easily accessible. Nevertheless, the potential role of gene therapy for the treatment of Alzheimer's disease and Parkinson's disease is currently the focus of intensive research. If gene transfer-cellular transplant approaches are effective, it will be possible to treat genetic, developmental, degenerative, infectious, or traumatic central nervous system dysfunctions.

Gene therapy applications have been proposed also for cancer treatment (28). In fact, deficiencies of cancer suppressor genes such as those apparently associated with retinoblastoma and Wilms' tumor could be treated by restoration of the expression of

the suppressor gene. Alternative approaches involve inactivation of dominantly acting oncogenes and antisense oligonucleotides to modulate the expression of oncogenes for the suppression of the cancer phenotype. The combination of gene transfer and cellular transplantation could become available to replace defective physiological products including hormones, serum proteins, and other metabolic products, in which transplantation of cells genetically modified could replace the compromised native sources.

Many diseases have been proposed as theoretical target for gene therapy, including disorders of serum proteins such as (hemophilia), hormone deficiencies such (diabetes mellitus) (34), and other enzyme or gene product-deficiency diseases, such as alpha(1)-antitrypsin deficiency.

<u>BONE MARROW TRANSPLANTATION</u>

Disorders that can be treated by bone marrow transplantation include severe combined immunodeficiency states, leukemias, various inherited disorders, osteoporosis, and solid tumors. A recent review on the subject has been written by Hardy and Ikpeazu (35). The first reported marrow transfusion was in 1939 to treat gold-induced aplasia (36). In 1957, Thomas reported the first series of human bone marrow recipients (37)-with transient positive results. Despite the advances in transplantation immunology and histocompatibility typing, the problems of rejection, graft-versus-host disease and infections still severely limit the success of this cellular transplant.

Development in molecular biology and genetic engineering may lead to new future applications.

STEM CELL TRANSPLANTATION

Hematopoietic progenitor cells, which are circulating in the blood can be isolated to replace marrow in certain circumstances in which a controindication for marrow harvest exists (38). Recently, human umbilical cord blood, that is usually discarded, have been used as a source of stem cells for clinical hematopoietic reconstitution (39), indicating that cord blood from a single individual could provide sufficient reconstituting cells for effective hematopoietic repopulation in an HLA-compatible allogeneic recipient.

NEURAL TRANSPLANTATION

Neural Transplantation to the central nervous system is at an early stage of development. The work has evolved towards two main goals: 1. promote regeneration or recreate damaged central neural circuits. 2. replacement of a particular chemical that is lacking in the recipient as a result of a lesion or a genetic disorder.

Neural transplantation has also attracted interest because of the reported prolonged survival of intracerebral grafts (23). Nevertheless, it became recently evident that even if cells can survive for prolonged periods after transplantation in this site, they survive in an immunologically unstable state and rejection can occur even after months of survival.

Future applications of neural transplantation in man include Parkinson's disease, to replace the degenerated dopamine-

containing input to the basal ganglia and Alzheimer's disease, in which the degeneration of the acethylcholine containing input to cerebral cortex and hippocampus could be corrected by implantation of fetal cholinergic brain cells into the cortex and/or hippocampus.

Fetal hypothalamic cell grafts could be used to treat deficiencies of hypothalamic releasing factors (41) such as in the case of transplantation of hypothalamic cells to the third ventricle of mice with testicular or ovarian/uterine hypotrophy resulting from a deficiency in hypothalamic gonadotropin releasing hormone (GnRH).

Finally, newborn rat retinal cells have been recently transplanted into an adult lesion site demonstrating integration and differentiation into the host retinal lesion site.

EPIDERMAL CELL TRANSPLANTS

Human epidermal cells from small skin biopsy samples have been recently used as a new source of autograft to treat patients with burns so extended that it is impossible to provide their complete coverage by skin grafts from the patient. In these cases epidermal cells can be cultured to produce epithelium sheets sufficient to cover the entire body surface (43-44). These cultured epithelial cells were initially used in the nude mouse model to demonstrate their ability to generate human epidermis after application to wounds (45). Their permanent epidermis was generated after transplantation on small burn wounds in adults and children (46-47). Successful treatment of

two children by cultured epithelial cells autografts to cover

burns on more than 95% of their bodies was finally reported in

1984 (48). The same technique for epidermal cell preparation has

been recently adopted in experimental neonatal epidermal cell

allotransplantation (49), in which indefinite survival of the

epidermal cell allografts was obtained across minor and major

histocompatibility barriers.

MYOBLAST TRANSPLANTS

The first attempts in human to correct Duchenne muscular

dystrophy by muscle cell transplantation have been performed in

the United States. In preliminary studies, muscle cells were

injected in the muscle of the foot which controls the movement of

a big toe of a 9 year boy affected by Duchenne muscular

dystrophy. An increase of 20% in the muscle strength was

announced. Several boys have been treated so far by muscle cell

transplant using cyclosporine as immunosuppressive agent, and

further studies are needed to determine the feasibility of this

approach.

HEPATOCYTE TRANSPLANTATION

Experimental hepatocyte transplantation indicated that it is

possible to retain hepatocellular functions after transplantation

of isolated hepatocytes (50). The possibility to correct

congenital enzyme deficiency diseases (51-53) in rodents and to

improve the survival rate in acute hepatic failure models (54-56)

by an hepatocellular transplants has also been observed. In

1989, Moscioni et al. (57) showed that human liver cells attached

to collagen coated microcarriers using a previous described

method (58-59), were able to function after transplantation into
mutant rat recipients which were genetically deficient in either
uridine diphosphoglucuronosyltransferase activity (Gunn rats) or
albumin synthesis (Nagase analbuminemic rats). The animals were
made genetically immunodeficient by interbreeding with athymic
rats so that transplantation of human tissue was made possible
without problems related to rejection. Interestingly the
injected hepatocyte microcarriers have been found on the surface
of the pancreas where newly formed blood vessels were present.
This finding has raised again the issue of hepatotrophic factors
and their role in the maintainence of hepatocytes transplanted in
ectopic sites. In fact, initial reports of proliferation of
hepatocytes transplanted into the spleen in the absence of direct
perfusion by portal blood and in the presence of an intact host
liver (50), were not confirmed in similar experimental conditions
by Cuervas-Mons et al. (60) who reported no evidence of
hepatocytes proliferation at any time after transplantation. In
addition, it has been reported (61) that in the absence of a
proliferative (70% of hepatectomy) in the recipient rats, it was
not possible to detect the hepatocytes at either two or four days
after intrasplenic transplantation. Cuervas-Mons et al. also
reported that there was no evidence of hepatocytes seven days
after allotransplantation in immunosuppressed rats (62). Cells
without nuclei and multinucleated cells were detected at the
transplant site, suggesting degeneration rather than rejection.
It may be that in the model of Demetriou et al. (58-59) of
collagen-coated microcarriers with attached hepatocytes the

microcarriers allow the survival of some of the hepatocytes. This may be due to a favorable effect of collagen matrix in the maintenance of hepatocyte in-vivo. The fact that hepatocytes-microcarriers aggregates were found on the surface of the pancreas repropose the role of factors produced from the pancreas in the maintenance of normal hepatocyte. To address this problem, a series of recent experiments both using rodents (63,64) and human (65) hepatocytes clearly demonstrated the protective effect of islets co-transplanted with hepatocytes in the maintenance of the ectopic hepatocytes. These reports demonstrated that it is possible to maintain hepatocytes in an ectopic site with an intact host liver present by addition of pancreatic islets to the hepatocellular grafts, while in the absence of pancreatic islets, degeneration of the hepatocytes occurred. The addition of collagen microcarriers of the same type as previously described by (57-59), did not improve human hepatocyte survival leaving only a thin rim of epithelioid cells attached to the collagen microcarriers (65). This experimental model is an example of combined cellular transplants in which a cellular population allows or improves the engraftment of a second cellular type. These results confirmed the pioneer work of Starzl that clearly established the role of splanchnic-derived hepatotrophic factors in the maintenance of hepatocyte integrity (66-69).

PANCREATIC ISLET TRANSPLANTATION

In 1893 Dr. Williams performed the first attempt of

pancreatic fragment graft in a fifteen year old diabetic child.
The discovery of insulin 29 years later determined an enormous
improvement in the care of diabetic patients, since it became
possible to control hyperglycemia and ketoacidosis, that were the
primary cause of death in these patients. Unfortunately, it
became apparent that the secondary complications of diabetes
which are now the primary cause of morbidity and mortality, have
not been prevented by exogeneous insulin therapy. Islet
transplantation has been proposed as an alternative treatment to
replace the endocrine pancreatic function in a more physologic
way. The procedures for isolation of the islet cells from the
exocrine tissue have been pioneered by the work of Moskalewski
(70) and Lacy (71). Lacy first introduced the concept of
intraductal distention of the pancreas and collagenase digestion
(71). A comprehensive review of the progress of experimental and
clinical islet transplantation is now available (72). The most
recent data of the islet transplant registry by Hering et al.
show that before 1984 no islet transplant demonstrated
significant islet function (C-peptide 1mg/ml) one month after
transplantation. Between 1985 and 1989 a significant improvement
in post-transplant islet function was observed. In fact, in over
thirty percent of the cases, basal C-peptide production was
observed in the first week post-transplant. But less than twenty
percent had documented c-peptide production at one month.

1990 was a critical year for islet transplantation because
of the demonstration of the ability of allogenic purified human
islets to achieve insulin independence after transplantation in

patients with pancreatectomy-induced type 1 diabetes. The first islet transplant of this series was performed in a fifteen year old girl who is still insulin independent 28 months following islet allotransplantation from a single donor (73). However, the preliminary trials of islet transplantation in Type 1 diabetic patients have been disappointing since only one patient is today still insulin free 2 years after allotransplantation of islets obtained from 5 donors. Several factors remain to be tested such as the adequacy of the implantation site, variables affecting islet engraftment, rejection, the diabetogenic effect of the immunosuppressive agents currently in use, and the role of the underlying autoimmune diseases.

CELL TRANSPLANTATION TO INDUCE DONOR SPECIFIC TOLERANCE

One of the most exciting developments in cell transplantation could be the possibility to use a cell transplant procedure to induce tolerance to cells, tissue and organs from syngeneic donors. Bone marrow transplantation has long been known to induce donor specific tolerance in recipient animals (74). Bone marrow is especially effective in its ability to confer tolerance to subsequent cellular, tissue or solid organ grafts from the same donor (75). Recently, we reported induction of donor specific tolerance to pancreatic islets following bone marrow transplantation in fully xenogeneic rat to mouse chimeras (76). It is of interest that donor type bone marrow derived antigen presenting cells, including dendritic cells, repopulated the recipients ubiquitously (77).

The phenomenon of cell migration and repopulation after transplantation is not exclusive in bone marrow transplants. In fact, donor dendritic cell migration into recipients has been observed following solid organ transplants such as heart, liver and intestine (78). The presence of donor antigen presenting cells in the recipients of bone marrow or solid organ transplants appears to be proportional to the amount of lymphoid and dendritic cells in the donor tissue and these cells are thought to be related to the facilitation of graft acceptance.

Studies are in progress to determine the relation between migration of donor antigen presenting cells, graft acceptance and the induction of donor specific tolerance. Co-transplantation of cells from bone marrow preparations may become in the future a requisite for tolerance induction to any allogeneic and xenogeneic transplant.

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