Cell transplantation for diabetes

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For 30 years there has been experimental work aimed at transplanting islets for the treatment of diabetes with a view to curing the disease and preventing the secondary complications. Many technical difficulties were experienced, first in isolating the islets without damaging them, and second in finding a suitable place to inject them, but until recently the results of a vascularized pancreas transplant have been superior to islet transplantation. In 2000, the group in Edmonton, headed by Shapiro, published encouraging results using a different immunosuppression in transplanting patients earlier in the course of their disease than had been attempted previously. The results were excellent at a year and good at 2 years in patients with Type I diabetes, however there was the rather worrying attrition at five years. Nevertheless, the Edmonton observations were proof of concept and have intensified interest in treating diabetes and other diseases where a specific protein synthesis was required by cell transplantation and/or genetic engineering. The recent interest in embryonic stem cells extenuated these efforts and progress is being made in defining the difficulties, which are greater than most workers would have predicted.

In this review, the subject is discussed explaining where progress needs to be made in order to provide treatment that would be of value to patients.

Keywords: current basis of transplantation; future prospects; historical review; genetic engineering; stem cells; diabetes

1. INTRODUCTION

To treat disease with cells is not a new concept. In the seventeenth century, before the nature of cell structures and functions were known, blood transfusion experiments were performed between animals using feather quill needles by Christopher Wren and his friends. For blood transfusions to be of value, rather than a 'Russian roulette' for sudden death, a means of preventing clot formation and an understanding of red blood cell groups were necessary. Then blood transfusions became life-saving and opened the door to modern major surgery.

In the 1950s, advances in immunology spearheaded by Peter Medawar and his colleagues revealed an immune system vital to life that could be manipulated by cell injection into animals *in utero* and allow acceptance of skin grafts from the cell donors, a process called 'acquired immunological tolerance' (Billingham *et al.* 1953).

An important advance in the treatment of haematological diseases followed from the demonstration that animals given 'lethal' doses of total body X-irradiation could be rescued by intravenous bone marrow infusions. The grafted bone marrow cells homed to the empty bone marrow spaces where the native marrow had been destroyed by the X-rays (Main & Prehn 1955). The donated marrow cells conferred on the recipients the immune characteristics of the donor. The closer the matching of the major histocompatibility complex

(MHC) between donor and recipient, the greater the likelihood of success.

More recently it has been possible to condition leukaemia patients to accept bone marrow grafts from well-matched donors without the need for complete destruction of the recipient bone marrow. This non-ablative treatment can result in mixed macro-chimaerism, with blood cells of both donor and recipient coexisting in the bone marrow and blood, so that it is possible to have the advantage of graft-versus-leukaemia immune reactivity, without excessively harsh treatment of the patient (Storb & McSweeney 1999). Moreover, this mixed chimaerism, even if only temporary, can result in kidney graft acceptance from the bone marrow donor (Juanita *et al.* 2004).

There is, therefore, a large literature and a long follow-up of clinical experience with therapeutic cell transplantation.

In the past 50 years, since the description of the double helical structure of DNA and an understanding of the mechanisms of protein synthesis, an accelerating advance in our knowledge of the molecular nature of many diseases has occurred. Many of the genes responsible have been identified and suggestions made as to how they might be used as engineering tools for therapeutic purposes.

The proliferation in culture of embryonic stem (ES) cells and the cloning of intact animals from adult somatic cells now create a challenge to provide cell therapy for many conditions that currently have inadequate treatment (Colman 2004).

2. DIABETES

There are two forms of diabetes, Type I and Type II, that differ in their pathogenesis.

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Type I is an autoimmune disease associated with certain genetic HLA configurations most commonly presenting between infancy and teenage years, but can also present in adults. Often, but not necessarily, the onset follows a viral infection and can be insidious. The β cells in pancreatic islets of Langerhans are singled out for immune destruction by primed T cells, whose molecular target has not yet been defined. Recovery of the β cell mass cannot occur due to continuing autoimmune activity and insufficient progenitor cells. Before the introduction of insulin in the 1920s, patients died, usually in a distressing, emaciated state, around puberty, before they could have children. Refinements in insulin therapy and a strict diet can restore patients to a relatively normal life, but even with excellent compliance to the regimen of frequent blood sugar estimations and insulin injections, the secondary complications of diabetes can develop in a relentless progressive manner causing blindness, renal failure, gangrene, coronary arterial disease and neuropathy. Inappropriate management of the therapeutic regimen can lead to dangerous and sometimes fatal hypoglycaemia, often with no warning for the patient. Insufficient insulin results in hyperglycaemic ketosis and diabetic coma.

The diagnosis of Type I diabetes in a child is a sentence to a lifelong strict regimen of diet and medication and is a major and continuing trauma to the whole family.

Type II diabetes is a common condition with many patients only mildly affected. The disease usually presents in adults but can present in children also. It is especially common in obese people and has reached almost an epidemic scale in India and South East Asia. Change from a frugal traditional diet to a liberal western-style of food has been blamed for the sudden increase in incidence of Type II diabetes in eastern countries. Initially many patients can be managed by diet and oral hypoglycaemic agents. Insulin resistance in the tissues is a feature of Type II diabetes, and the β cell mass may increase producing excessive amounts of insulin apparently in an attempt to overcome the resistance. Eventually, there is β cell failure and in approximately half of the cases exogenous insulin injections are necessary and the same secondary complications occur as in Type I diabetes.

Taken together, Type I and Type II diabetes result in serious morbidity and mortality in all communities. Diabetes is a major cause of blindness and renal failure. In addition to the cost in human suffering, the financial burden of diabetes on health care resources is enormous and accelerating yearly as the incidence of both Type I and Type II diabetes increases.

(a) *The role of insulin*

The history of insulin is fascinating and has been told especially well by Michael Bliss in *The Discovery of Insulin* (Bliss 1982).

In 1889, Minkowski & Von Mering in Strasbourg found that dogs subjected to pancreatectomy became diabetic. One account of the finding was that the technician raised the suspicion of sugar in the urine to Minkowski, by observing flies settling in large numbers on the puddles of urine passed by the diabetic dogs, in

contrast to their relative lack of interest in the urine of normal dogs.

In 1869, Paul Langerhans, a medical student writing his thesis, observed microscopic islands of different structure to the main mass of digestive enzyme secreting pancreas. This seminal observation, perhaps the most perspicacious of any medical student, led to intense study of the islets. They are miniature organs embedded within the pancreas in most creatures, but constituting separate independent organs in some fish. Each islet consists of approximately 1000 cells of four distinct types each with its own secretion task:

α cells produce glucagon

β cells produce insulin. They constitute 60–80% of the cells in the islets i.e. 6–800 cells/islet

δ cells produce somatostatin

pp cells produce pancreatic polypeptide

There is a delicate and profuse capillary network and nerve connections in the islet, somewhat resembling the renal glomerulus. The islet can be considered as a mini-organ. The capillaries of the islets anastomose with the main pancreatic vasculature, which may facilitate signalling between endocrine and exocrine pancreatic cells. The interaction of cytokines between the individual cell types may be important attributes that would be lost to separated islets or surrogate β cells. The pancreas contains approximately one million islets and, therefore, $6-8 \times 10^8$ β cells. The endocrine secretions of the islets enter the portal blood and the first organ they reach is the liver. Insulin is partially metabolized by the liver which converts glucose to glycogen.

In the 1920s, the connection between removal of the pancreas and diabetes was established, but attempts at treatment with various oral preparations of pancreas did not ameliorate diabetes. The young orthopaedic surgeon, Frederick Banting, working in Toronto, was convinced that an extract of pancreas injected would provide the vital substance missing in diabetes. With the technical assistance and a major intellectual contribution from a medical student, Charles Best, the two rather low profile researchers produced an extract of pancreas that lowered the blood sugar of diabetic dogs, and eventually in 1922, they persuaded clinical colleagues to try a similar extract in diabetic patients. Some but not all of the early clinical cases responded but first the help of a protein chemist, James Collip, was needed. There was much opposition from conservative clinicians, but eventually the concept was accepted that a substance from the pancreatic islets called 'insulin' could be used as a treatment for diabetic patients. It soon became apparent that a large commercial pharma company, with deep pockets and prepared to accept a risky project, would be required to produce enough of the substance in relative purity to provide lifelong treatment. The Eli Lilly Company stepped in, rose to this challenge, and the lives of diabetics were transformed, albeit with the reservations of the diabetic way of life and the risk of complications to which I have referred.

The molecular structure of the complicated protein insulin was determined in Cambridge in the 1950s at the Laboratory of Molecular Biology by Frederick Sanger in the course of his first Nobel Prize work. The physiology of insulin and the control of glucose metabolism is complex. Before active insulin is available, a non-active molecule called C-peptide must be cleaved from the parent molecular proinsulin. There is an important basal secretion of insulin, but on the intake of food, insulin granules, stored in the β cells, are released in a pulsatile manner simultaneously from a number of β cells, in amounts relating to the ambient blood glucose concentration in the islets. The timing is critical. If released too early or too late, high insulin blood levels will cause inappropriate, possibly dangerous, hypoglycaemia. If not enough insulin is available at the appropriate time, normal glucose metabolism cannot take place and the blood sugar level will rise. There is a considerable reserve of β cell function, so after even a large meal not all the β cells exhaust their supply of secreted insulin from within their cell membranes. There is a slow turnover of β cells, perhaps around 5% per annum in man, from progenitor cells present in the islets and/or in the ducts of the exocrine pancreas. In rodents the turnover is much greater (Bonner-Weir 2000).

The chemistry of insulin secretion varies in different species. In man, as stated above, an inactive proinsulin is the first main synthetic step and this becomes cleaved into the inactive C-peptide, a marker of insulin synthesis and active insulin. In mice there are two active insulins, I and II. In diabetic patients, the level of glycosolated haemoglobin in the blood rises. The interactions between insulin, glucagon and other endocrine secretions are complicated and in some patients microangiopathy develops in the retinae, glomeruli and small blood vessels throughout the body, associated with serious complications.

First passage of insulin through the liver is physiological, but release of insulin directly into the caval venous system appears to be well tolerated following vascularized pancreatic transplants.

(b) *Vascularized pancreas transplantations*

Surgical transplantation of a vascularized whole pancreas or even half a pancreas can give excellent long-term results (Sutherland 1997), with cure of diabetes in many cases. Most patients have suffered from diabetic renal failure and often it has been possible to transplant a kidney and a pancreas from the same donor. Powerful lifelong immunosuppression is necessary, but this would be standard treatment for the kidney graft. The operation is a major surgical procedure with the special danger of leakage of pancreatic digestive enzymes, but results are improving steadily. Unfortunately the incidence of diabetes is far in excess of the availability of donor pancreata.

(c) *Islet cell transplantation*

Islets when separated are small enough to survive temporarily in a suitable environment, by simple diffusion of nutrients and oxygen into them and CO₂ and waste products out, whilst a new blood supply is established. The idea of transplanting islets based on

the same concepts as split skin grafts is an old one; islets however do not part company with their surroundings in the pancreas easily. In rodents they can be hand-picked under a dissecting microscope, but in large animals including man enzymatic digestion and mechanical chopping of the pancreas are necessary. The islets are vulnerable to damage from ischaemia and the effects of collagenase and the more refined enzyme 'liberase'. Dicing the pancreas into small pieces also damages the islets. An elaborate highly skilful and prolonged process is necessary. Five people working for 5 h, with a cooled pancreas removed immediately from a brain-dead cadaver may, in the best circumstances, produce about 3–400 000 or 1/3 of the total number of islets in a tolerably well-preserved state suitable for transplantation. Yet twice that number is required to release a patient from the need for insulin injections. The islet isolation procedure has some fanciful resemblance to digging for potatoes on a dark night with a sharp spade.

The next unanswered questions are:

- (1) Should the islets be cultured before transplantation?
- (2) Can they be safely frozen and thawed?
- (3) Most importantly, where to transplant them.

In mice, an artificial space under the kidney capsule is a good site to inject islets despite the caval drainage of insulin. In man, the portal blood stream has been most favoured, the islets hopefully lodging as microemboli in the liver sinusoids, where they take up residence and after a few days acquire a new blood supply, mainly from recipient capillaries growing into the transplanted islets. Islets floating in the blood are in an abnormal environment and may activate complement causing local platelet aggregation and clot formation precluding rapid neovascularization and endangering liver parenchyma to ischaemia (Bennet *et al.* 2000; Goto *et al.* 2004). An optimal site for islet transplantation has yet to be found, in the meantime the report of clinical islet transplantation by Shapiro *et al.* (2000) in Edmonton has marked a halt to the extensive scepticism that prevailed in the transplant community for clinical islet grafting.

Using usually two cadaveric pancreas donors per recipient and immunosuppression designed to try and avoid diabetogenic toxicity, the Edmonton workers obtained 80% independence at 1 year from the need for exogenous insulin and 70% at 2 years in Type I diabetics with brittle disease, usually involving hypoglycaemic unawareness, but without other serious diabetic complications. Repeating their results has only been possible in a few of the specialized centres that have made the attempt.

Unfortunately there is progressive attrition of the grafted islets, only 50% of transplanted patients being free of the need for insulin injections after 5 years. The mechanism of the deterioration is not known but could be a mixture of slow rejection, recurrence of the autoimmune disease or exhaustion of the β cells. Auto-transplants of islets from pancreata removed for chronic pancreatitis can do well long-term. In such

cases there would be no allograft rejections nor autoimmune disease.

The shortage of suitable human cadaveric pancreata and the huge numbers of diabetics would make it reasonable to view the Edmonton experience as an extremely important 'proof of principle' that the procedure is possible, but at great cost of healthcare resource and skilled technical ability, with the lucky patients no longer requiring insulin, but nevertheless having to take full doses of immunosuppressive drugs indefinitely. No doubt better yields of islet extraction will be achieved and safer immunosuppressions developed, but the disadvantages outlined above remain.

(d) *Xeno-islet grafting*

Pig insulin differs from human insulin only in one amino acid. Porcine insulin has been used successfully therapeutically in patients for many years. Porcine glucose homeostasis is similar to man and pig islets are potentially available in large numbers and can be extracted in a similar manner to that used for human islets. The pig however is a different species, separated from man in evolution by many millions of years, and of the hundreds or even thousands of proteins produced by pig cells, each is different to the human equivalent and most are capable of eliciting immune destructive reactions following transplantation.

To date results of xeno-islet transplantation to primate species have been disappointing, but Bernard Hering has recently obtained encouraging results of pig to monkey islet grafts using powerful immunosuppression with agents that could be used in patients (Wijkstrom *et al.* 2003). The question again arises does the immunosuppression justify the procedure? There are worries that porcine endogenous retrovirus might cause disease. There are hopes that genetic engineering of pigs by 'knock out' and 'knock-in' genes to make pigs more like humans or at least make their tissues more acceptable as grafts to man may one day be successful, but how soon cannot be predicted. Many transplant researchers have sympathy with Norman Shumway's comment "xenografting is the future of organ transplantation and always will be!"

(e) *Other approaches*

- (1) Large-scale proliferative culture of β cell progenitor cells in pancreatic ducts or from islet β cells. This is attractive in that these are the cells that normally produce β cells, but to date there has been a severe shortfall in numbers of β cells that can be produced in culture and so far the numbers are far below the threshold of therapeutic use (Bonner-Weir *et al.* 2000); also the site of origins of the precursor cells is disputed (Dor *et al.* 2004).
- (2) Transdifferentiation of liver cells to islet cells. Both liver and pancreas develop from the same embryological rudiment so, by the use of certain growth factors and cultural procedures, workers have succeeded in taking this step in experimental settings (Alam & Sollinger 2002; Ber *et al.* 2003; Horb *et al.* 2003; Nakajima-Nagata *et al.* 2004).

- (3) *In vivo* 'cultural' growth of embryonic pancreas rudiments. Hammerman in St. Louis (Hammerman 2001) and Reissner in Israel (Dekel *et al.* 2003) have achieved considerable progress in this endeavour but any clinical application would seem to require an excessively costly 'bespoke' individual approach for each patient. The use of foetal tissue would raise worrying ethical dilemmas (Castaing *et al.* 2001). The justification of using a foetus to treat a patient with diabetes might be difficult to sustain.
- (4) Guide or engineer undifferentiated or differentiated cells to act as surrogate β cells.

(i) *Embryonic stem cells*

Since ES cells can and do turn into every cell type in the body, their use for producing β cells has received much publicity and Soria has been successful in introducing the human insulin gene into mouse ES cells and selecting the cells producing insulin to treat diabetic mice successfully (Soria *et al.* 2000).

This was an important achievement, but may be difficult to translate in the context of human ES cells, which grow more slowly and are more vulnerable to death in culture than murine ES cells. Monkey ES cells have been differentiated into pancreatic cell phenotypes (Lester *et al.* 2004).

If an *in vitro* process using human ES cells was successful, it would be of vital importance to eliminate every undifferentiated cell from the inoculum to be given to patients because of the risk that such cells might differentiate into teratomata (Soria 2001).

Somatic nuclear transfer to egg cells could produce bespoke stem cells isologues to those of the patient. This approach is still in its infancy and would be very expensive but in theory would avoid the need for immunosuppressive drug treatment.

(ii) *Adult 'stem cells'*

Multipotent cells have been identified in a number of adult tissues and in umbilical cord blood. They are the source of successful bone marrow grafts and may have the potential to differentiate into other cell lineages, though such claims are disputed.

Blood monocytes have been shown to de-differentiate under certain cultural conditions, into cells which can be persuaded with growth factors and certain cultural conditions to proliferate some five to sixfold and then differentiate into liver like cells producing albumen, islet-like cells producing insulin and glucogen and fat cells or return back to monocytes (Abuljadayel 2003; Zhao *et al.* 2003; Ruhnke *et al.* 2005).

If sufficient insulin-producing cells could be obtained from a specimen of the patient's blood by plasmaphoresis, the return of these cultured cells now producing insulin should not, in theory, elicit an immune reaction. They are autologous and presumably would be unlikely to have the autoimmune target of Type 1 diabetes, although this has yet to be established.

In experiments recently reported, monocytes were isolated from human peripheral blood and treated with

M-CSF and IL-3 for 6 days to induce a state of plasticity (Ruhnke *et al.* in press). They were then exposed to an islet differentiation medium containing EGF, HGF and nicotinamide for 4–8 days. Small clumps of cells developed in culture resembling islets. These neo-islets exhibited pancreas-specific gene expression by RT-PCR, immunocytochemistry, and radioimmunoassay. In addition, the neo-islets were transplanted to streptozotocin-induced diabetic mice.

Transplanted animals retained normal blood glucose levels for up to 8 days ($n=5$), when these xenograft human monocytes were rejected since the animals were not treated with immunosuppression. These encouraging results, if repeated, would indicate an attractive approach of cell therapy using autologous cells. Important questions are raised: (1) could enough cells be obtained from the diabetic patients? (2) would the neo-islets behave physiologically for a useful period? (3) Are the cultural procedures and reagents used safe?

(iii) *Transfecting adult cells with the human insulin gene with a glucose sensing promoter*

This approach can use non-viral electroporation to introduce the insulin gene plasmid into cells *in vitro* or *in vivo*, with encouraging experimental results using adult liver cells (Chen *et al.* 2005). Alternatively viral vectors can be used which are more efficient, but some viruses have the danger of unmasking oncogenes (McCormack & Rabbitts 2004).

Viral vectors. One of the main attributes of virus behaviour is to gain entry into target cells and either reside there or kill the cells, having made use of their nuclear material. To act as a vector the virus must be big enough for the construct in question. Most studies have been with two classes of virus - the adeno and adeno-like viruses and the lente-modified HIV retro viruses. Early clinical trials of both classes have sometimes led to modest clinical improvement, but three disasters have been reported. In one case in Philadelphia the adeno virus proliferated with fatal consequences (Marshall 1999; Raper *et al.* 2003). In the other two cases in Paris it would appear that the lente virus used had unmasked nuclear oncogenes leading to leukaemia (McCormack & Rabbitts 2004; 'episome'). These tragedies have alerted researchers to the dangers and have also led to sharp and often aggressive criticism of the workers. Despite this background in the foreseeable future cultural techniques alone may not be sufficient and vector help may be needed.

Currently we are working with a modified herpes I virus as a vector for the human insulin gene. A variant of this virus has been used as local treatment for glioblastoma and injected into the brain. There has been no evidence of systemic disease in the six patients treated with a follow-up of 5 years (Rampling *et al.* 2000).

The theoretical advantages of the herpes I virus are:

- (1) the large capacity to accommodate a construct;
- (2) the ability of the virus to infect primary and second cell lines *in vitro*;
- (3) although the virus enters the nucleus it does not integrate with the host DNA and is therefore not likely to unmask oncogenes. It functions separate to the host DNA as an episome;
- (4) most patients have already had contacts with the herpes I virus, which normally resides in a quiescent state in neurological tissue;
- (5) immune reaction against the virus is relatively mild;
- (6) established antiviral treatment against the herpes virus is available.

We and others are engaged in experiments to determine which cell line or tissue might be appropriate for engineered viral infection and whether it is preferable to work *in vitro* with autologous cells to be returned to the recipient or should the virus be injected directly into recipient tissue. We need to study the longevity of gene activity in the virus and what factors may limit its continued protein synthesis.

The hope of large scale cell treatment of diabetes may still be a long way from fulfilment, but the intensity of research along the lines suggested above makes the hope at least a possibility in the eyes of an optimist.

REFERENCES

- Abuljadayel, I. S. 2003 Induction of stem cell-like plasticity in mononuclear cells derived from unmobilised adult human peripheral blood. *Curr. Med. Res. Opin.* **19**, 355–375. (doi:10.1185/030079903125001901.)
- Alam, T. & Sollinger, H. W. 2002 Glucose-regulated insulin production in hepatocytes. *Transplantation* **74**, 1781–1787. (doi:10.1097/00007890-200212270-00024.)
- Bennet, W. *et al.* 2000 Damage to porcine islets of Langerhans after exposure to human blood *in vitro* or after intraportal transplantation to cynomolgus monkeys. *Transplantation* **69**, 711–719. (doi:10.1097/00007890-200003150-00007.)
- Ber, I., Shternhall, K., Perl, S., Ohanuna, Z., Goldberg, I., Barshack, I., Benvenisti-Zarum, L., Meivar-Levy, I. & Ferber, S. 2003 Functional, persistent and extended liver to pancreas transdifferentiation. *J. Biol. Chem.* **278**, 31 950–31 957. (doi:10.1074/jbc.M303127200.) Epub May 29, 2003.
- Billingham, R. E., Brent, L. & Medawar, P. B. 1953 Actively acquired tolerance of foreign cells. *Nature* **172**, 603–606.
- Bliss, M. 1982 *The discovery of insulin*. USA: University of Chicago Press.
- Bonner-Weir, S. 2000 Life and death of the pancreatic beta cells. *Trends Endocrinol. Metab.* **11**, 375–378. (doi:10.1016/S1043-2760(00)00305-2.)
- Bonner-Weir, S., Taneja, M., Weir, G. C., Tatarkiewicz, K., Song, K. H., Sharma, A. & O'Neil, J. J. 2000 *In vitro* cultivation of human islets from expanded ductal tissue. *Proc. Natl Acad. Sci. USA* **97**, 7999–8004. (doi:10.1073/pnas.97.14.7999.)
- Castaigne, M., Peault, B., Basmaciogullari, A., Casal, I., Czernichow, P. & Scharfmann, R. 2001 Blood glucose normalization upon transplantation of human embryonic pancreas into beta-cell-deficient SCID mice. *Diabetologia* **44**, 2066–2076. (doi:10.1007/s001250100012.)
- Chen, N. K. F., Sivalingam, J., Tan, S. Y. & Kon, O. L. 2005 Plasmid-electroporated primary hepatocytes acquire quasi-physiological secretion of human insulin and restore euglycemia in diabetic mice. *Gene Ther.* **12**, 665–667.

- Colman, A. 2004 Making new beta cells from stem cells. *Semin. Cell Dev. Biol.* **15**, 337–345. (doi:10.1016/j.semcdb.2004.02.003.)
- Dekel, B. *et al.* 2003 Human and porcine early kidney precursors as a new source for transplantation. *Nat. Med.* **9**, 55–60. (doi:10.1038/nm812.)
- Dor, Y., Bornbw, J., Martinex, O. I. & Melton, D. A. 2004 Adult pancreatic β -cells are formed by self-duplication rather than stem cell differentiation. *Nature* **429**, 41–46. (doi:10.1038/nature02520.)
- Goto, M., Johansson, H., Maeda, A., Korsgren, O. & Nilsson, B. 2004 Low molecular weight dextran sulphate prevents the instant blood-mediated inflammatory reaction induced by adult porcine islets. *Transplantation* **77**, 741–747. (doi:10.1097/01.TP.0000114872.26990.4F.)
- Hammerman, M. R. 2001 Growing kidneys. *Curr. Opin. Nephrol. Hypertens.* **10**, 13–17.
- Horb, M. E., Shen, C. N., Tosh, D. & Slack, J. M. 2003 Experimental conversion of liver to pancreas. *Curr. Biol.* **13**, 105–115. (doi:10.1016/S0960-9822(02)01434-3.)
- Juanita, M. S., Tatsuo, K. & Yasuhiro, F. 2004 Mechanisms of donor-specific unresponsiveness in tolerant recipient of combined non-myeloablative HLA-mismatched bone marrow and kidney transplantation. *AMJT* **4**(Suppl. 8), 303. (abstract no. 526.)
- Lester, B. L., Kuo, H. C. & Andrews, L. 2004 Directed differentiation of rhesus monkey ES cells into pancreatic cell phenotypes. *Reprod. Biol. Endocrinol.* **2**, 42. (doi:10.1186/1477-7827-2-42.)
- Main, J. M. & Prehn, R. T. 1955 Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J. Natl Cancer Inst.* **15**, 1023.
- Marshall, E. 1999 Gene therapy death prompts review of adenovirus vector. *Science* **286**, 2244–2245. (doi:10.1126/science.286.5448.2244.)
- McCormack, M. P. & Rabbitts, T. H. 2004 Activation of the T-cell oncogene LM02 after gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* **350**, 913–992. (doi:10.1056/NEJMra032207.)
- Nakajima-Nagata, N., Sakurai, T., Mitaka, T., Katakai, T., Yamato, E., Miyazaki, J., Tabata, Y., Sugai, M. & Shimizu, A. 2004 *In vitro* induction of adult hepatic progenitor cells into insulin-producing cells. *Biochem. Biophys. Res. Commun.* **318**, 625–630. (doi:10.1016/j.bbrc.2004.04.059.)
- Ramplung, R. *et al.* 2000 Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 171) in patients with recurrent malignant glioma. *Gene Ther.* **7**, 859–866. (doi:10.1038/sj.gt.3301184.)
- Raper, S. E., Chirmule, N., Lee, F. S., Wivel, N. A., Bagg, A., Geo, G. P., Wilson, J. M. & Batshaw, M. L. 2003 Fatal systemic inflammatory response syndrome in an ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol. Genet. Metab.* **80**, 148–158. (doi:10.1016/j.ymgme.2003.08.016.)
- Ruhnke M. *et al.* 2005 Reprogramming of human peripheral blood monocytes into functional hepatocyte and pancreatic islet-like cells. *Gastroenterology*. (In press.)
- Ruhnke M. *et al.* In press. Normalization of blood glucose by human monocyte-derived pancreatic islet-like cells (Neo-islets) in streptozotocin diabetic mice. *Transplantation*.
- Shapiro, A. M., Lakey, J. R., Ryan, E. A. & Korbitt, G. S. 2000 Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **343**, 230–238. (doi:10.1056/NEJM200007273430401.)
- Soria, B. 2001 *In vitro* differentiation of pancreatic beta cells. *Differentiation* **68**, 205–219. (doi:10.1046/j.1432-0436.2001.680408.x.)
- Soria, B., Roche, E., Berna, G., Leon-Quinto, T., Reig, J. A. & Martin, E. 2000 Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* **49**, 157–162.
- Storb, R., Yu, C. & McSweeney, P. 1999 Mixed chimerism after transplantation of allogeneic hematopoietic cells. In *Hematopoietic cell transplantation* (ed. E. D. Thomas, K. G. Blume & SJ Forman) 2nd ed., p. 287. Boston: Blackwell.
- Sutherland, D. E. R. Newsletter Int. Pancreas Transplant Reg. 9:1
- Wijkstrom, M., Kirchhof, N. & Clemmings, S. 2003 Prolonged pig islet xenograft survival and function in cynomolgus monkeys immunosuppressed with basiliximab, Rad and FTY720. *Transplantation* **76**. (abstract no. 75)
- Zhao, Y., Glesne, D. & Huberman, E. A. 2003 Human peripheral blood monocyte-derived subset acts as pluripotent stem cells. *Proc. Natl Acad. Sci.* **100**, 2426–2431. (doi:10.1073/pnas.0536882100.)