Transplantation: focus on kidney, liver and islet cells

Edward N. Chang, MD, MSc; Charles H. Scudamore, MD, MSc; Stephen W. Chung, MD, PhD

Over the past decade, advances in immunosuppression, organ preservation, surgical techniques and perioperative management have resulted in improved survival rates for solid organ transplants. Even so, the field of transplantation still presents many challenges. A critical obstacle is the shortage of donor organs. The paucity of cadaveric organs has increased the demand for living donor transplantation. Although this option has expanded the organ pool, concerns over ethical issues and donor safety remain, and there is an ongoing effort to make living donation a safer and less invasive process. An alternative to solid organ transplantation involves the transplantation of cells, such as islet cells for type 1 diabetes mellitus. Whereas transplantation of solid organs has seen steady improvement over the past 2 decades, transplantation of islet cells has not. Recent advances in the field of islet cell transplantation, however, have made this procedure a clinical reality. Stem cell research has provided a glimpse into the possible future of transplantation for organ failure. Another major barrier to transplantation is the lifelong need for immunosuppression. Current immunosuppression protocols place transplant recipients at continuing risk for immunosuppression-associated complications such as infection and malignant disease. New agents continue to reduce the rates of acute graft rejection and to increase long-term survival; however, they have exposed metabolic and cardiovascular complications without affecting the incidence of chronic rejection. The ultimate goal of many investigators in this field is to achieve specific immunologic graft tolerance. In this article we summarize recent technical advances in the field of transplantation that address some of the challenges.

Au cours de la dernière décennie, les progrès de l'immunosuppression, de la préservation des organes, des techniques chirurgicales et de la prise en charge périopératoire ont entraîné un amélioration des taux de survie à la suite de la transplantation d'organes solides. Malgré cela, le domaine de la transplantation pose toujours de nombreux défis. La pénurie d'organes de donneurs constitue un obstacle critique. La rareté des organes de cadavre a fait grimper la demande de transplantation d'organes de donneurs vivants. Même si cette option a augmenté l'offre d'organes, des questions d'éthique et la sécurité des donneurs préoccupent toujours et l'on s'efforce continuellement de rendre le don d'organes de donneurs vivants plus sûr et moins effractif. La solution de rechange à la transplantation d'organes solides consiste à transplanter des cellules, par exemple d'îlots pancréatiques dans le cas du diabète de type 1. La transplantation d'organes solides s'est améliorée régulièrement au cours des deux dernières décennies, mais ce n'est pas le cas de la greffe des cellules d'îlots. Des progrès récents réalisés dans le domaine de la greffe de cellules d'îlots pancréatiques ont toutefois concrétisé cette intervention sur le plan clinique. La recherche sur les cellules souches a soulevé le voile sur l'avenir possible de la transplantation en cas de défaillance d'organes. L'obligation pour le receveur de prendre des immunosuppresseurs pendant le reste de sa vie constitue un autre obstacle majeur à la transplantation. Les protocoles d'immunosuppression en vigueur exposent les receveurs à un risque continu de complications associées à l'immunosuppression comme les infections et les tumeurs malignes. De nouveaux agents continuent de réduire les taux de rejet aigu des greffons et de prolonger la survie à long terme, mais ils entraînent des complications métaboliques et cardiovasculaires sans avoir d'effet sur l'incidence du rejet chronique. Beaucoup de chercheurs dans ce domaine visent en bout de ligne à produire une tolérance immunologique spécifique du greffon. Dans cet article, nous résumons les progrès techniques réalisés récemment dans le domaine de la transplantation qui permettent de s'attaquer à certains des défis.

From the Department of Surgery, University of British Columbia, the Vancouver General Hospital and the British Columbia Transplant Society, Vancouver, BC

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Correspondence to: *Dr. Stephen W. Chung, Department of Surgery, Vancouver General Hospital, 910 West 10th Ave., Vancouver BC V5Z 4E3; fax 604 874-4036; stchung@interchange.ubc.ca*

Transplantation of solid organs is a relatively new field that emerged in the latter half of the 20th century. The first human kidney transplantations were performed in the 1930s. Without any knowledge of transplantation immunology or organ preservation techniques, it is not surprising that all were unsuccessful, mostly because of graft rejection. Subsequently, the discovery that allograft loss in human skin transplant models was due to a recipient-generated immune response established the scientific foundations of transplantation. However, there was still no means of modulating this immune reaction. Although the first successful kidney transplant between identical twins was made by Murray (who subsequently received the Nobel Prize for Medicine in 1990) and Hume in 1954,[1] most attempts at human kidney transplantation in the early 1950s failed. Despite these disappointing clinical results, breakthroughs in research later in the decade, such as cellular immunity and the role of lymphocytes in allogeneic transplantation, allowed greater understanding of the processes involved with graft rejection. Concurrently, the discovery of human histocompatibility antigens and the implementation of pre-transplant lymphocytotoxic crossmatching underlined the importance of proper donor–recipient immunologic matching.

With a better understanding of transplant immunology, investigators began to focus on ways to depress the recipient's immune system. The earliest successful immunosuppression combination of azathioprine and prednisone resulted in prolonged survival of human renal transplants, and by the mid-1960s this regimen emerged as the standard for post-transplantation immunosuppression. This immunosuppression regimen was augmented subsequently by the discovery of antilymphocyte globulin and monoclonal antibody therapy.[2]

Clinical outcomes in renal transplantation were further improved by the discovery that warm ischemia had detrimental effects on the donor organ. This highlighted the importance of in situ perfusion and storage of grafts in specially designed preservation solutions such as the Collins and EuroCollins solutions. The biochemical composition of these solutions attempted to mimic an intracellular environment and minimize cellular swelling. These solutions have now been supplanted by the University of Wisconsin and histidine-tryptophan-ketoglutarate (HTK) solutions that permit longer cold preservation of the organs.

It was not until cyclosporine was introduced into immunosuppressive regimens in the late 1970s and early 1980s that the modern era of transplantation began. Cyclosporine is a potent inhibitor of T-lymphocyte function and was found to greatly improve outcomes in renal transplantation. This served as the impetus for surgeons to attempt technically more challenging procedures such as heart, lung, liver and pancreas transplantation. The mainstay of immunosuppression therapy today remains directed at the inhibition of the molecular pathways of T-cell activation and function. The drugs used include calcineurin inhibitors (tacrolimus and cyclosporine) that interfere with the interleukin-2 gene activation protein NF-AT (nuclear factor of activated T cells), corticosteroids (prednisone) that interfere with T-cell growth factors and antigen presentation, and agents that interfere with T-cell proliferation (azathioprine and mycophenolate mofetil, which are antimetabolites, and sirolimus, which inhibits targets of rapamycin). Many transplant recipients also receive antilymphocyte induction therapy to immobilize their existing T cells. These agents include polyclonal antibody preparations such as antilymphocyte globulin and antithymocyte globulin, or monoclonal antibody preparations such as OKT3. Recently, more specific biological inhibitors of T-cell function have been introduced. They include the anti-interleukin-2 receptor antibodies basiliximab and daclizumab, agents that are now in clinical use. Immunosuppressive regimens that eliminate steroid use and reduce calcineurin inhibitors are being developed by combining existing medications with newer agents, such as the immunodepleting agent alemtuzumab (Campath-1H). The aim of these new regimens is to optimize recipient immunosuppression while minimizing the deleterious side effects of the drugs.

Liver transplantation

The chronic shortage of donor organs, particularly in children, has prompted innovations that will maximize the benefit from each available cadaveric organ and expand living donor transplantation. Over the last decade, procedures such as cadaveric split-liver transplantation, reduced-size liver grafts, adult–pediatric and adult–adult living-related transplants have been developed in attempts to augment the donor organ pool.[3]

Split-liver transplantation

The use of a single donor liver for 2 recipients was first reported from Germany in 1988.[4] To ensure adequate hepatic mass, the right lobe (segments V–VIII) is usually transplanted into an adult recipient, with the left lateral portion (segments II–III) reserved for either a child or a small adult. Although traditionally performed on the back table, a few groups have recently demonstrated improved outcomes with in situ splitting at the time of organ retrieval. This has the advantage of minimizing cold ischemia time and improving hemostasis of the cut liver surface. However, because of the longer retrieval process, in situ splitting should not be performed on unstable donors, and the procedure itself may elicit hemodynamic instability.

Recently, Yersiz and colleagues[5]

published a review of 100 consecutive in situ split-liver transplantations performed between 1991 and 2003. Outcomes and complication rates for these transplantation procedures were compared to those for living donor and cadaveric whole-organ recipients during the same period. The 100 split-liver procedures netted 190 grafts (97 left-lateral-segment and 93 right-lobe grafts) that were transplanted into both pediatric and adult patients of all clinical statuses. Although there was no significant difference in the rates of biliary and vascular complications in recipients of left-lateral-segment grafts when compared with living donor and cadaveric whole-organ recipients, there was a higher rate of primary nonfunction and a trend toward poorer survival, but the latter finding was not statistically significant. Recipients of in situ split right-lobe grafts had similar complication and survival rates to those of living-donor recipients. The authors concluded that split-liver transplantation remains a potentially important means for expanding the donor pool. Although it has yet to gain widespread acceptance, split-liver transplantation continues to be practised at specialized centres, especially in Europe.

Adult living-donor liver transplantation

This has been an important advance in expanding the donor pool. The procedure was first established in children in 1990,[3] and the first adult case was reported in 1994.[6] In children and small adults, transplantation of segments II and III (the left lateral segment) is usually adequate, whereas in adults, the right lobe (with or without the middle hepatic vein) is usually transplanted to provide sufficient hepatic mass. Advantages of living-donor liver transplantation include the following: waiting time is markedly reduced (the recipient may receive a transplant as soon as an appropriate donor is identified and assessed); the elective nature of the operation permits optimal preparation of the recipient preoperatively; the potential for organ damage from cadaveric organ donor retrieval is reduced; and cold ischemia time is reduced because the harvested organ can be inserted immediately. Over the last decade, the number of adult living-donor liver transplantations performed in the United States has increased significantly (Fig. 1).[7]

However, there are still many unanswered questions concerning adult living-donor liver transplantation. First, since this is still a relatively new field, long-term donor and recipient outcomes are largely unknown. Brown and associates[8] recently conducted a large survey of this procedure in adults at all transplantation centres in the US (449 procedures from 42 centres). Of the recipients, 22% had biliary complications and 9.8% had vascular complications. Others have reported biliary complication rates ranging from 15% to 32%,[9] findings that indicate a higher rate of these complications for recipients who receive partial liver grafts from living donors than those who undergo standard cadaveric whole-liver transplantation. In contrast, overall survival for short-term grafts and liver recipients seems to be comparable for those receiving cadaveric whole-liver transplants and living-donor partial liver grafts (Fig. 2).[7]

The major controversy surrounding living-donor liver transplantation stems from the risk to the donor. Brown and associates[8] reported an overall donor complication rate of 14.5%, with bile stricture or leak (8.5%) being the most common complication. Although they reported only 1 donor death, in the US, donor mortality is estimated at 0.3%, with 3 reported perioperative deaths.[10] Two other donors required transplantation after hepatic resection had resulted in inadequate hepatic mass.[11] The European Liver Transplant Registry reported a 0.8% donor mortality in 2000.[12]

Umeshita and colleagues[13] reported on 1800 living donors in Japan between 1989 and 2002. There were no perioperative deaths, and the overall donor complication rate was 12%. The incidence of complications was significantly higher in donors who underwent a right-sided resection than in those who donated a left lobe or left lateral segment. Biliary fistula, gastric stasis and wound infection were the most common complications, and 1.2% of the donors required reoperation. The mean hospital stay for donors was 15.6 days.

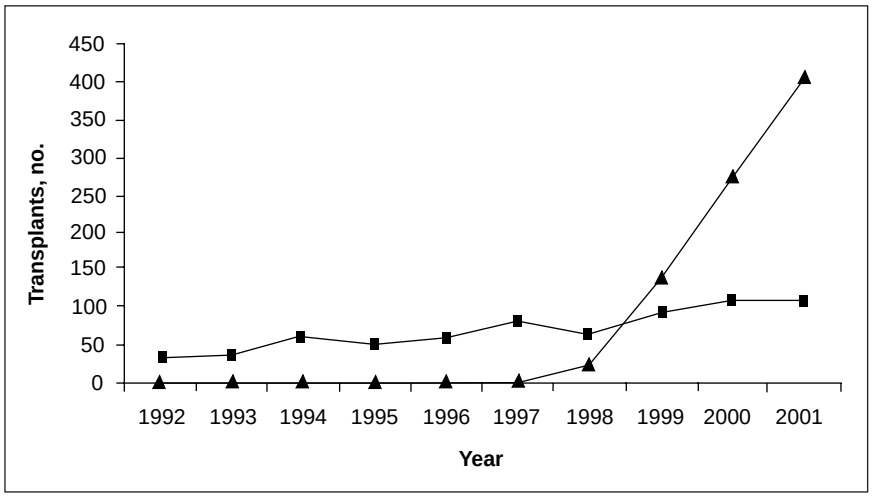

FIG. 1. Living-donor liver transplantations performed in the United States in children (< 18 yr) (squares) and adults (≥ 18 yr) (triangles) between 1992 and 2000. Source: 2002 OPTN/SRTR annual report.

Kidney transplantation

Laparoscopic donor nephrectomy

First performed in 1995,[14] the laparoscopic donor nephrectomy has evolved from an experimental procedure to being the standard of care for kidney procurement at many major centres for living-donor renal transplantation. The 2 main controversies surrounding laparoscopic donor nephrectomy are similar to those for living-donor liver transplantation (i.e., donor safety and outcome of both graft and recipient). When compared with open donor nephrectomy[15] and "mini-incision" donor nephrectomy,[16] the laparoscopic approach is associated with a shorter hospital stay and time to return to preoperative activity, less patient discomfort and lower overall costs.[17,18] As a consequence, the procedure has increased the overall donor pool by making kidney donation more appealing to the general population.[19]

Improved surgical techniques and instrumentation as well as increased experience with the procedure have addressed initial difficulties of ureteral dissection and preservation of adequate vascular length. Concerns over donor safety have also been addressed. Large series have shown that laparoscopic nephrectomy is associated with less blood loss and fewer complications than open procedures.[18,20] Hand-assisted laparoscopic donor nephrectomy is another procedure that is becoming increasingly popular. Its advocates report that it is less technically demanding, more easily mastered and thus more widely applicable than standard laparoscopy.[15,18] Furthermore, the hand-assisted approach may be associated with shorter operating times, warm ischemia time and lower operative risks than standard laparoscopic donor nephrectomy.[21]

Another unanswered question pertains to the outcomes of grafts procured laparoscopically. Clinicians have always been aware of the prolonged warm ischemia time for laparoscopic nephrectomy. However, the effect of this on graft function was largely unknown. Troppmann and colleagues[22] reviewed the United Network for Organ Sharing (UNOS) database and compared recipient and graft outcomes from 2743 laparoscopically procured grafts and 2576 grafts procured through an open approach. They found that laparoscopic nephrectomy may be associated with delayed graft function. However, the 1-year acute rejection rates and graft survival rates were similar for both groups. Although delayed early graft function has been associated with poorer long-term outcomes, no study has yet compared long-term graft outcome of laparoscopic versus open donor nephrectomy.

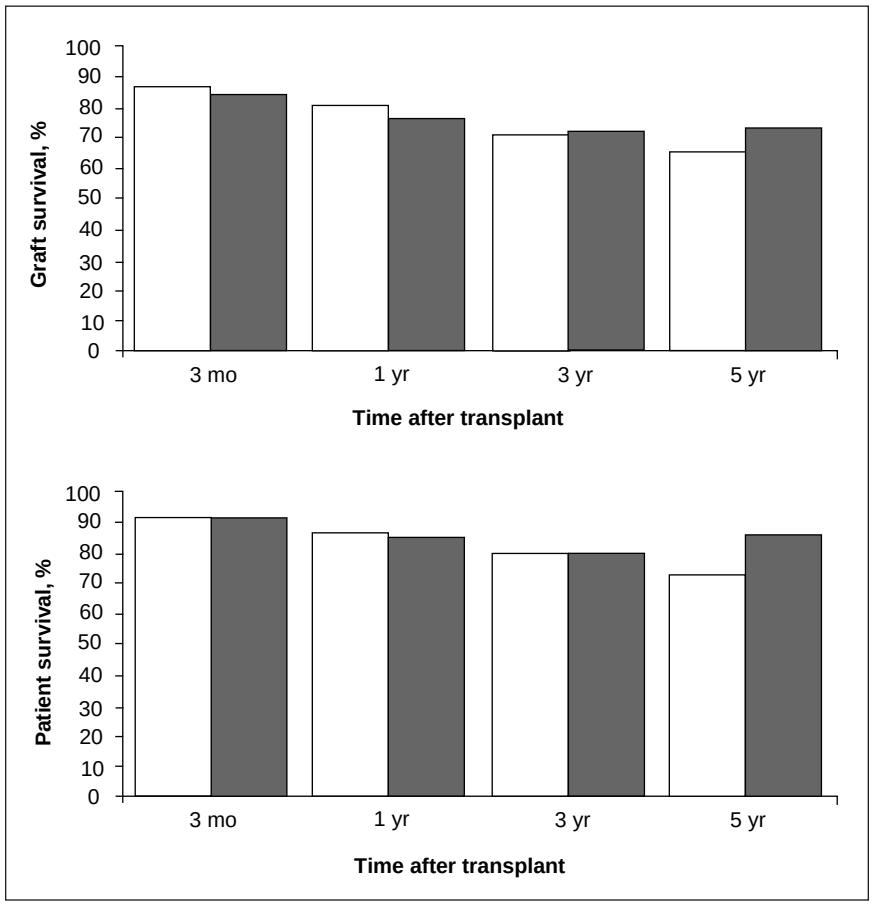

FIG. 2. Graft survival (top) and patient survival (bottom) for liver transplant recipients (living (black columns) v. dead (white columns) donors). Source: 2002 OPTN/SRTR annual report. Cohorts are for transplants performed during 1999–2000 for 3-month and 1-year, 1997–1998 for 3-year and 1995–1996 for 5-year survivals.

Islet transplantation

Allogeneic islet transplantation

The introduction of the Edmonton Protocol resulted in renewed interest in clinical allogeneic islet cell transplantation.[23] Major components of the protocol include transplanting an adequate number of high-quality islets, appropriate recipient selection and individualized immunosuppression (Table 1). As of November 2002, 41 patients had received islet transplants with use of the Edmonton Protocol, with insulin independence being achieved in 82% of patients 1 year after transplantation (Fig. 3).[23]

Despite these encouraging outcomes, there are still numerous challenges to be faced before islet trans-

plantation becomes widely available. First, the demand for islets greatly outweighs the supply, a problem being attributable to the limited number of potential donors. This predicament is compounded by current difficulties in purifying large numbers of islets from cadaveric pancreases. At present, it takes an average of 2 donors to supply enough islets to transplant into 1 recipient. Second, the enhanced immune response elicited against the graft is a major obstacle in islet transplantation. Not only are the islets besieged by "normal" host immune cells, which recognize the allogeneic graft as foreign, but they also face an "abnormal" host autoimmune response that is already primed to attack islets. The literature on islet autotransplantation has demonstrated that insulin independence is achievable with a much smaller transplanted islet mass when both host allo- and autoimmune responses are absent. Therefore, to minimize the number of islets required by each recipient (thereby eliminating the need for multiple donors) and circumvent the need for immunosuppression, research is also focused on means to make the transplanted islets undetectable by the host's immune system.

A promising "immunoisolation" technique involves coating the islets with a water-soluble, semipermeable membrane or microcapsule. Studies have shown that encapsulated islets can regulate glucose homeostasis while evading the host immune response. Large animal models are being studied to determine the most favourable location for engraftment of encapsulated islets. Recently, intraportal microcapsule injection in a porcine model was found to produce similar hemodynamic, biological and radiologic results as human islet transplantation.[24]

Stem cell transplants

Another means of addressing the shortage of islets is to find alternative sources of insulin-producing β cells. Both embryonic and adult stem cells have been considered as potential sources for β cells. The principal distinctions between these 2 types of stem cells are their respective sources and differentiation capacities. Human embryonic stem cells are harvested from the inner cell mass of 4- or 5-day-old blastocysts that have been fertilized in vitro. They have the capacity to differentiate into any cell of the body when the appropriate stimulus is applied (i.e., they are pluripotent). Although these embryonic stem cells appear to be the ideal source for cell replacement therapy, there are ethical considerations that require resolution. Conversely, adult stem cells are undifferentiated cells that are found within differentiated tissues. In general, adult stem cells can only specialize into the specific cell types of the tissue from which they originated.

Pancreatic ductal and acinar cells as well as islet cells themselves have been studied as potential sources of

Table 1

Major Components of the Edmonton Protocol for Allogeneic Islet Cell Transplantation

Component	Requirements
Sufficient number of high-quality pancreatic islets	Refined pancreas procurement techniques Improved enzyme digestion and purification process Multiple donors as needed for adequate islet-cell mass
Recipient selection	"Brittle" type 1 diabetic patients Normal renal function, no severe cardiovascular disease No insulin resistance, moderate insulin requirements
Tailored immunosuppression	Sirolimus-based Steroid-free Induction with anti-interleukin-2-receptor antibody Low-dose calcineurin inhibitor

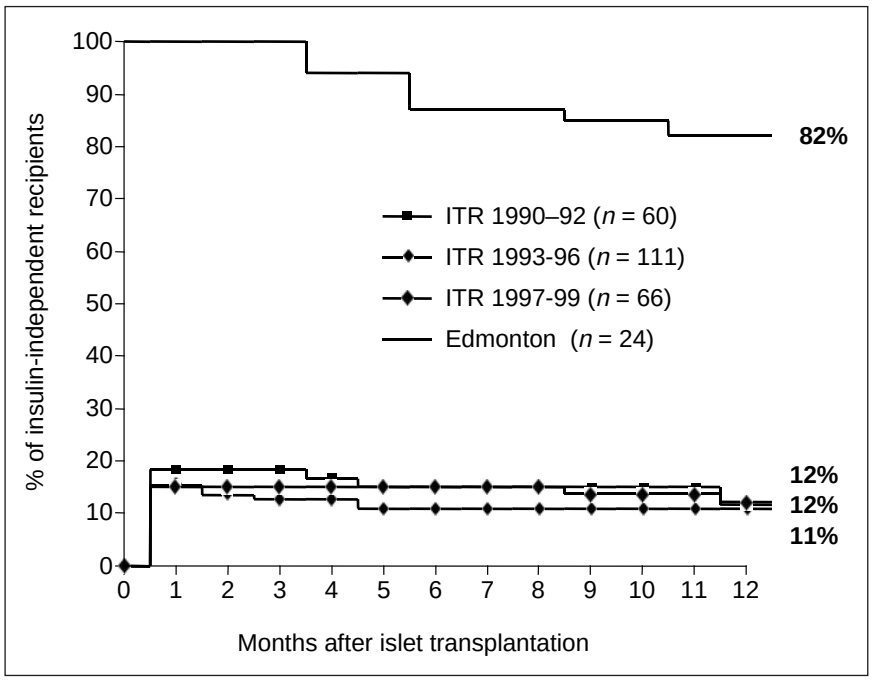

FIG. 3. Cumulative 1-year insulin independence after allogeneic islet transplantation in type 1 diabetic patients for the international Islet Tumour Registry (ITR) and the Edmonton Protocol. Adapted and reproduced by permission from Oberholzer J, Shapiro AM, Lakey JR, Ryan EA, Rajotte RV, Korbutt GS, et al. Current status of islet cell transplantation (review). *Adv Surg* 2003;37:253-82.

adult pancreatic stem cells. Recently, it has been shown that human pancreatic duct cells could be converted into insulin-secreting cells by genetic engineering techniques.[25] Unfortunately, insulin release in these cells was low and not regulated by glucose levels. Also, there was a question of whether these cells were true β cells or "insulin-producing cells." Thus, at present, the exact identity and location of adult pancreatic stem cells remain elusive.

In contrast, although glucose-responsive insulin-containing cells have been generated from mouse embryonic stem cells,[26,27] further experiments suggested that the majority of them may have obtained their insulin content by absorption from the surrounding culture media, and only rarely did these cells transcribe the insulin gene.[28] Nevertheless, when grafted into diabetic mice, these cells appeared to regulate glucose homeostasis and prevent death.[27]

Much work is still needed before functional glucose-responsive β cells can be derived from stem cells, and, since whole islets comprise not only β cells but also α, δ and γ cells, it is uncertain whether β-cell replacement alone will be sufficient to achieve insulin independence in diabetic patients.

Islet autotransplantation

Patients with benign pancreatic disorders (mainly end-stage chronic pancreatitis), who require complete or partial surgical resection of the pancreas, are at increased risk for insulin-dependent diabetes mellitus postoperatively. Although harvesting the islets from the surgical specimen and transfusing them back to the patient in an attempt to prevent insulin dependence is not a new concept, the procedure is not widely available. Brendel and associates of The International Islet Transplant Registry[29] reviewed outcomes from all reported cases of islet autotransplantation between 1990 and 2000. They found that 47% of patients who underwent autotransplantation remained insulin independent at 1 year (a rate of 71% if > 300 000 Islet Equivalents were transplanted). In general, the duration of insulin independence correlated directly with the number of islets transplanted. Long-term (> 13 yr) insulin independence after resection of the pancreas has also been reported.[30]

A critical factor in determining the yield and quality of harvested islets is the quality of the pancreatic tissue. Currently, pancreatic resection is only offered to those with end-stage chronic pancreatitis in whom the organ is almost entirely fibrotic. Outcomes of islet autotransplantation likely would be greatly improved if these patients underwent the procedure when the disease was in its early stages. Early pancreatic resection is not an unreasonable option since these patients all have borderline glucose homeostasis, and in approximately two-thirds of them, type 1 or type 2 diabetes will develop due to progressive pancreatic destruction.[31] Furthermore, the recent successes of allogeneic islet transplantation have led many new centres to initiate islet transplantation programs and have resulted in the discovery of better methods of islet processing. These factors may make islet autotransplantation after total or near-total pancreatectomy a more widely available and successful means of preventing diabetes postoperatively in patients with benign pancreatic disorders.

Immunologic advances

Fifty years ago, Billingham and associates[32] reported their discovery of immunologic tolerance. Since then, much research has been devoted to discovering ways to induce tolerance, although none have yet been proven broadly applicable to the clinical setting. Hence, immunologic tolerance remains the "Holy Grail" of transplantation research. Clinically, tolerance can be defined as "immune unresponsiveness in the absence of ongoing therapy to graft alloantigens but not to other (third party) antigens."[33] A variety of techniques have been shown to induce immunologic tolerance. These can be broadly classified into 2 main categories: co-stimulatory molecule blockade and immunologic ablation with hematopoetic reconstruction.

Co-stimulatory molecule blockade

Co-stimulatory molecule blockade involves inhibiting specific signals between cells of the immune system. T-cell activation and proliferation require the presence of numerous co-stimulatory signals between the T cell and the antigen-presenting cell. T cells that encounter antigen in the absence of these signals become tolerant of that antigen.

In large-animal models, co-stimulatory molecule blockade with a variety of non-depleting monoclonal antibodies in the peritransplant period resulted in tolerance of the allograft. For example, perioperative administration of an antibody against CD154 (CD40 ligand) has been shown to prevent acute rejection of kidney transplants in rhesus monkeys.[34,35]

Regulatory T cells

Co-stimulatory molecule blockade may work through a subset of T cells called regulatory T cells (T_{regs}). These cells constitute 5%–10% of all peripheral CD4+ T cells and function mainly to suppress self-reactive T cells that have escaped central clonal deletion in the thymus. If left unregulated, these self-reactive T cells could mediate harmful autoimmune responses in the host. Interestingly, in addition to protecting against autoimmune diseases, T_{regs} can induce a state of tolerance in animal models of allogeneic transplantation. T_{regs} harvested from mice that were made tolerant to allogeneic skin grafts via molecule blockade were able to abrogate graft rejection when infused into identical mice that had not been

rendered tolerant to their skin grafts.[36] Experiments demonstrated that the transfused T_{regs} mediated tolerance through suppression of naïve host T cells. In turn, these "regulated" naïve T cells were able to suppress other populations of naïve T cells, thereby permitting long-term graft acceptance.[37]

Immunoablation and chimerism

The other major strategy for inducing tolerance is ablation of the recipient's immune system followed by reconstitution with donor hematopoietic stem cells. The ablation can be achieved with radiation (total body or thymic) or with immunosuppressive agents. The recipients become hematologic chimers (i.e., they have the hematologic make-up of 2 genetically different individuals) and can accept further allografts from the same donor without any need for immunosuppression. Long-term survival of allogeneic kidney grafts without immunosuppression has been reported in human recipients with hematologic malignant disease who had received allogeneic bone marrow transplants from the same donor.[38,39] Unfortunately, given the great risks associated with allogeneic bone marrow transplantation (i.e., infection and graft-v.-host disease), this approach to achieve chimerism is not clinically applicable for most transplantation candidates. Nevertheless, some encouraging results have been achieved using less aggressive ablation protocols in humans. Recently, Mathew and colleagues[40] reported improved graft survival and a lower rate of chronic rejection in immunosuppressed cadaveric kidney transplant recipients who had received donor bone marrow infusions than in recipients who had not.

Conclusions

The ongoing critical shortage of donor organs places an emphasis on appropriate selection of potential transplant recipients and optimization of their post-transplant care to maximize the lifespan of each transplanted organ. Expansion of the living donor pool will require continuing technical innovation and improved safety for the donors. Alternative attempts to address end-stage organ failure, such as the use of stem cell technology, face many hurdles, but significant steps have already been taken. Inducing immune tolerance remains the "Holy Grail" of transplantation. There has been a paucity of clinically applicable tolerance-inducing regimens, but the use of bone marrow and stem cell infusions with careful immunosuppression induction therapy shows promise for the future.

Competing interests: None declared.

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