Chapter 2
Islet and Pancreas Transplantation

Davide Mineo, Gaetano Ciancio, George W. Burke, Rodolfo Alejandro, and Camillo Ricordi

Abstract  Islet allotransplantation for patients with brittle type 1 diabetes mellitus (T1DM) is a minimally invasive and relatively safe procedure that can induce sustained, normalized glucose control and restore C-peptide secretion, with reduction of hypoglycemic episodes, stabilization or delay of chronic complications, and better quality of life. Current immunosuppressive protocols have significantly improved short-term outcomes, whereas long-term results are still inadequate (from 80% to 10% insulin-independence from 1 to 5 years post-transplant). Principal limitations include: imperfections in the islet isolation process, auto- and alloimmunity, allosensitization, immunosuppression-related toxicity, and unsuitability of the intrahepatic implantation site. More efficient isolation methods, safer and more efficient immunosuppressive agents in tolerogenic strategies, and alternative transplant site(s) may resolve these limitations in the near future. Simultaneous pancreas–kidney (SPK) transplantation is the optimal treatment for patients with T1DM with end-stage renal disease. Restoration of normoglycemia after pancreas transplant, as well as of renal function after kidney transplant, results in significant improvement of neuropathy, retinopathy, and nephropathy. Novel immunosuppressive therapies, improvements in surgical techniques, and better understanding of postoperative recipient care have improved results of SPK transplants consistently over the past decade. Future directions include optimization of immunosuppression, allowing freedom from insulin injection therapy while maintaining normoglycemia, and avoidance of chronic transplant glomerulopathy, with durable normalization of kidney function, thus improving quality of life as well as extending patient survival.

2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is a cell-specific autoimmune disease triggered by environmental factors (e.g., viral infections, toxins, diet nutrients or antigens) in genetically predisposed individuals [e.g., human leukocyte antigen (HLA)]
class II DR/DQ, insulin-VNTR, and CTLA4 genes], primarily children and young adults. This chronic process leads to selective destruction of the insulin-producing \(\beta\) cells within the pancreatic islets. The resultant complete deficit of insulin, the main hormone regulating glucose as well as lipid and protein metabolism, causes hyperglycemia, which leads to acute (ketoacidosis) and chronic (retinopathy, nephropathy, neuropathy) complications, hypercoagulability, dyslipidemia, and accelerated atherosclerosis, with poorer quality of life, increased cardiovascular disease, and reduced life expectancy (The Diabetes Control and Complications Trial Research Group 1993; Zimmet et al., 2001; Leroith et al., 2003).

T1DM represents 5–10% of all cases of DM. It is estimated that in 2010 the worldwide prevalence of T1DM will be 0.1–0.5% of the general population, more than 6 million patients (1 out of 100–300 newborns), and its incidence will be 30–50 new patients per each 100,000 individuals, with a 3% increase yearly, mainly in developing nations acquiring a western lifestyle and diet. In addition to racial and regional differences involving the genetic background and environmental triggers, possible reasons for such an increase in T1DM frequency are the rise in childhood obesity and increasing sedentary lifestyle, which cause metabolic stress by development of insulin resistance and inflammatory injury to \(\beta\) cells with functional exhaustion, thus accelerating the onset and progression of the disease (accelerator theory) (The Diabetes Control and Complications Trial Research Group, 1993; Zimmet et al., 2001; Leroith et al., 2003; Yoon and Jun, 2005; Daneman, 2006; Wilkin, 2008).

T1DM-related micro- and macro-vasculopathy are the main causes of blindness, chronic end-stage renal disease requiring dialysis, and peripheral limb amputations and deformities, together with associated disabilities, comorbidities, and death. Their impact involves some 10% of total health-care expense in Western countries, with over $100 billion spent every year in United States and over $200 billion worldwide. Daily exogenous insulin is the treatment of choice in association with tailored diet and physical exercise programs. Novel insulin formulations (e.g., glargine and lispro analogues) together with infusion-pump and glucose-sensor technologies have significantly improved metabolic control, with lower rates of side effects, prevention or reduction of chronic complications, and better quality of life (The Diabetes Control and Complications Trial Research Group 1993; Zimmet et al., 2001; Leroith et al., 2003; Daneman, 2006).

### 2.2 Pancreatic Islet Allotransplantation

Intensive insulin treatment in T1DM has been associated with increasing severe hypoglycemic episodes, which can associate with cardiovascular accidents and deterioration of glucose control. Up to 10–20% of long-standing T1DM patients cannot achieve stable metabolic control or avoid life-threatening hypoglycemia and progressive complications, owing primarily to diabetic neuropathy with hypoglycemia unawareness and a concomitant alteration of the contraregulatory mechanisms.
Attempting tight glycemic control is of major importance in view of the high mortality rate among such subjects while they wait for over 4 years for a pancreas transplant. In this subgroup of T1DM patients, β-cell replacement therapy by allogeneic pancreatic islet transplantation (IT) might be an attractive, less invasive, and safer option than pancreas transplantation. Despite the fact that it improves glucose control, chronic complications, and quality of life, and provides longer graft survival and function, pancreas transplantation has a higher risk of perioperative morbidity and mortality (The Diabetes Control and Complications Trial Research Group 1993, 1997; Zimmet et al., 2001; Grussner et al., 2004; Grussner and Sutherland, 2005; Ryan et al., 2006; Lipshultz and Wilkinson, 2007; Gerstein et al., 2008; McCrimmon, 2008).

The pancreatic islets of Langerhans, which contain the insulin-producing β cells, are functionally complex endocrine structures that detect minimal changes in blood levels of glucose and other metabolites and maintain metabolic homeostasis through a fine real-time secretion of specific hormones. IT is an alternative therapy that can restore physiological glucose sensing and insulin delivery in patients with unstable T1DM (Cabrera et al., 2006; Leibiger and Berggren, 2008).

Clinical indications for IT include T1DM patients with basal or stimulated C-peptide of less than 0.3 ng/ml and imminent or current end-stage renal disease who will receive a kidney transplant, namely simultaneous islet–kidney (SIK) transplants from the same donor, or who already had a kidney transplant and will receive an islet-after-kidney (IAK) transplant from a different donor. IT alone (ITA) is a valid option for T1DM patients with normal or minimally altered renal function and frequent acute and severe metabolic complications requiring urgent medical care (e.g., life-threatening hypoglycemic episodes, severe hyperglycemia, or recurrent ketoacidosis); and/or with incapacitating physical and emotional problems with insulin therapy; and/or with failure of insulin management to prevent chronic complications (Ryan et al., 2006; Marzorati et al., 2007).

The main goal of IT is to achieve stable, normalized glycemic control and absence of severe hypoglycemic episodes, thus improving quality of life, preventing long-term diabetic complications, and reducing procedure- and immunosuppression-related side effects. Insulin independence, although desirable, is not necessarily the primary goal of IT, although a significant reduction in insulin requirements and the restoration of C-peptide secretion are desirable and have some beneficial effects (Ryan et al., 2006; Leitao et al., 2008a).

### 2.2.1 Islet Transplantation Procedures

#### 2.2.1.1 Recipient and Donor Selection

Selection of IT recipients is based primarily on the following criteria: patients who have had T1DM for at least 5 years, are 18–65 years of age, with a body mass index (BMI) less than 26 kg/m², and have one or more of the following conditions: severe, incapacitating hypoglycemic episodes with lack of awareness (based on Clarke or
Hypo scores); poor, labile glucose control [according to mean amplitude glucose excursion (MAGE) or lability index], with hemoglobin-A1c (HbA1c) greater than 8.0% despite intensive insulin therapy and care; and progressive diabetic complications. Exclusion criteria include: nephropathy [creatinine > 1.6 mg/dl, estimated glomerular filtration rate (eGFR) < 80 ml/min, and albuminuria >300 mg/24 h], unstable diabetic retinopathy or neuropathy, or any condition limiting islet engraftment and survival or immunosuppression (e.g., hepatitis) (Ryan et al., 2004, 2006; Marzorati et al., 2007).

Criteria for selection of multiorgan, brain-deceased, and heart-beating donors include: subjects of 25–45 years of age, with BMI greater than 25 kg/m², and negative record or evidence of DM or other severe or chronic illness, transmissible infective agent or disease, under toxic substance or drug abuse. Several donor characteristics may positively influence the outcomes of the isolation process and the islet yield: age 16–40, BMI > 27, male gender; traumatic death; normoglycemia while hospitalized; use of steroids and vasopressors, especially pitressin, with hemodynamic stability; shorter duration of cardiac arrest and hypotension; and a larger organ size with surface integrity and no edema (Lakey et al., 1996; Nano et al., 2005; Ryan et al., 2006; Marzorati et al., 2007; Ponte et al., 2007; Hanley et al., 2008).

Donor–recipient ABO compatibility is required, together with negative lymphocyte cross-match and panel reactive antibody (PRA) of less than 20%. In SIK, HLA-matching is quite strict in order to guarantee kidney graft survival, whereas in ITA and IAK histocompatibility is not required. This strategy, although limiting the recurrence of autoimmunity, which relies on intrinsic β-cell antigenicity, increases the risk of HLA-dependent allorejection (Roep et al., 1999; Bosi et al., 2001).

2.2.1.2 Pancreas Procurement, Islet Isolation, and Transplantation

The pancreatic islets of Langerhans are tight mixed clusters of different endocrine cells scattered throughout the pancreas, each type secreting a specific hormone: α cells (glucagon), β cells (insulin and amylin), δ cells (somatostatin), and PP cells (pancreatic polypeptide). It is estimated that the number of islets in a normal human pancreas is about 1 million, but significant variations can occur depending on donor age, sex, or weight and organ size and integrity (Ricordi, 1992; Leroith et al., 2003; Cabrera et al., 2006; Leibiger and Berggren, 2008).

The islet isolation process is designed to obtain an adequate yield of integral and functional islets from donor pancreata. Pancreas procurement and preservation are key steps for a successful isolation, requiring a short (<10 min) warm ischemia time (interval between uncontrolled non-heart-beating up to resumption of heart activity), organ recovery by an expert surgical team (preferably from the same IT program), pancreas storage in standard iced-chilled preservation solution, and short (<12 h) cold ischemia time (interval between pancreas harvesting and the islet isolation) (Ricordi, 1992; Lakey et al., 1996; Lee et al., 2004; Ponte et al., 2007; Porrett et al., 2007; Hanley et al., 2008).

Despite an increase in organ donations, rates of pancreas recovery remain unsatisfactory and much lower than those of other solid organs. Indeed, from more than
8000 multiorgan donors available in the United States in 2006, only 2000 pancreata were recovered, and only 1440 were used for transplantation, with the remaining not being retrieved because of poor organ and donor quality (63%, mainly owing to altered exocrine and/or endocrine function), placement-related issues and time constraints (9%), or other undefined causes (28%). Furthermore, IT centers receive a pancreas only after it has not been accepted for whole organ transplantation at the local, regional, or national level, often when the cold ischemia time has exceeded the ideal. A recent pancreas allocation scheme attempts to minimize this time, placing organs from donors over 50 years or BMI of more than 30 kg/m\(^2\) directly for IT, but it may include older subjects with reduced islet function and mass or borderline diabetics with higher islet mass but lower insulin secretion capacity. A poor utilization of potential “islet donor pancreata” has also been reported. In fact, in the United States in the period 2000–2004, from the overall pool of pancreata available, only 22.3% were used for whole organ transplantation (“optimal” glands); of the remaining ones, 48.5% were considered “suitable islet donors” (11% “optimal” and 89% “standard”), but only 2.1% of them (only 8.7% of the “optimal” donors) were used for IT. There is a wide margin for improvement in pancreas allocation and utilization, including the use of “optimal” donors and a fair noncompetitive organ distribution between IT and pancreas transplantation programs, which might fulfill the demand of the small T1DM population requiring \(\beta\)-cell replacement (Lakey et al., 1996; Deng et al., 2004; Ihm et al., 2006; Porrett et al., 2007; Stegall et al., 2007; Hanley et al., 2008).

A semiautomated method of mechanically enhanced enzymatic pancreas dissociation in a digestion-dissociation chamber (Ricordi chamber), with different blends of lytic enzymes (e.g., collagenases and proteases), is used to release the islets from the surrounding interstitial-connective and exocrine tissues. A semiautomated purification technique in a computerized centrifuge system (COBE 2991), with various density gradient solutions (e.g., glucose-based), is performed thereafter to separate the endocrine from the exocrine cells (Fig. 2.1). Finally, a small volume (<5 ml) of highly purified islet product is recovered and undergoes a 2-day culture for cell recovery from the traumatic isolation process. The cell culture medium is enriched with trophic and antioxidant substances (e.g., insulin, nicotinamide, L-glutathione) to prevent oxidative stress and apoptosis, preserving \(\beta\)-cell function and survival (Ricordi et al., 1988; Ricordi, 1992; Ichii et al., 2006).

This interval also allows for assessment of islet survival, content, and function prior to transplantation, thus determining product clinical suitability by FDA-approved tests. Islet counting is performed at optical microscope from final product samples using diphenylthiocarbazone (DTZ) staining (selectively binding to zinc–insulin granules with red coloring). The islet mass is calculated using an algorithm whereby islets are scored according to their diameter and counted as the number of islet equivalents (IEQ) based on a standard islet size of 150 \(\mu\)m. Product purity is evaluated as a percentage of DTZ-stained endocrine cells compared to unstained exocrine cells. Islet viability is assessed by fluorescent inclusion–exclusion dyes selectively binding to viable or necrotic cells. Islet function is determined in vitro by measuring glucose-mediated insulin release in static incubation
(low-then high-glucose challenge) and expressed as a stimulation index (SI, ratio of stimulated-to-basal insulin release). A decision for transplant is made when sufficient islets are recovered (minimum 350,000 IEQ, or 5000 IEQ/kg of recipient body weight) and specific product release criteria are met: endocrine tissue > 30%; islet viability > 70%; SI value > 1; negative Gram stain; endotoxins levels < 5 EU/kg (Ricordi, 1992).
Despite significant progress, even in the most experienced centers fewer than 50% of the pancreata processed with the intent to transplant provide a sufficient number of islets; moreover, more than 50% of the pancreatic islet content is lost in the process, as a result of donor brain-death related events, suboptimal organ preservation, deficient isolation process, and inadequate β-cell cytoprotection. Overall, these conditions limit the chances of a satisfactory islet yield from a single pancreas, so that frequently more than one donor is required to collect the number of islets needed to normalize glucose control or achieve insulin independence (Nano et al., 2005; Pileggi et al., 2006, Ponte et al., 2007).

IT occurs via microembolization into the hepatic portal venous system, with islet entrapping in the peripheral branches, at the presinusoid level due to size restriction, followed by their engraftment and revascularization from the hepatic vasculature, with immediate function and sustained survival. The transplant is performed by gravity infusion from a closed-bag system containing the heparinized islet product in the main portal vein through percutaneous transhepatic catheterization, under fluoroscopic and ultrasound guidance, using local anesthesia and conscious sedation, with close monitoring of portal pressure. This minimally invasive interventional radiologic procedure lasts approximately 1 h and patients are discharged from the hospital within 24–48 h, once clinically stable and if no complications arise. In SIK, or if there are contraindications to this approach (e.g., risk of hemorrhage, anatomical anomalies), cannulation of a tributary of the portal vein, such as the mesenteric or umbilical vein, is performed by laparotomy or laparoscopy (Baidal et al., 2003; Pileggi et al., 2006; Goss et al., 2003).

2.2.2 Clinical Protocols

2.2.2.1 Historical Protocols

Following the first case of a functioning allogeneic IT reported in 1980, several trials in patients with T1DM were performed in late 1980s, mainly as SIK and IAK or in combination with other solid organ transplants. Variable numbers of pancreatic islets, purified from cadaver single-donors, were injected into the liver during the main organ transplant or through a transient intraportal catheter as a post-transplant percutaneous procedure. The immunosuppressive regimens were those traditionally used in solid organ transplants, combining corticosteroids (prednisolone or methylprednisolone), purine antagonist azathioprine or calcineurin inhibitor (CNI) cyclosporine A, with lymphodepleting polyclonal antibodies added at induction in a few trials [e.g., diverse animal-derived antithymocyte globulin (ATG)] (Largiadr et al., 1980; Mintz et al., 1988).

The first promising results in IT were reported in the context of multiorgan transplants in the early 1990s using the new CNI tacrolimus, with greater immunosuppressive effect and fewer side effects than cyclosporine A, as a maintenance drug. Later on, mycophenolate mofetil (MMF), a prodrug of mycophenolate acid (MPA), a purine synthesis inhibitor, was launched as a maintenance drug with equal
immunosuppressive efficacy but lower nephrotoxicity than CNI. At the same time, more efficient induction strategies were tested, and the two monoclonal antibodies daclizumab and basiliximab, targeting the IL2 receptor/CD25 on T-lymphocytes with functional and proliferative inhibition, were used with significant reduction of acute rejection episodes. In contrast, muromonab-OKT3, targeting the T-cell surface marker CD3 with profound lymphodepletion, was tested but soon abandoned owing to severe cytokine release. In a few trials, bone marrow cells (BMCs) or hematopoietic stem cells (HSCs) from the same single-donors were coinfused, using lymphodepleting nonmyeloablative conditioning, in the attempt to induce recipient hematopoietic chimerism and islet graft tolerance, but islet graft survival was not maintained after discontinuation of immunosuppressive drugs (Tzakis et al., 1990; Ricordi et al., 1992; Gores et al., 1993; Alejandro et al., 1997; Secchi et al., 1997; Oberholzer et al., 2000; Pileggi et al., 2004).

The overall results of this first decade of IT trials were encouraging but not satisfactory, and limited islet graft survival, high rates of primary nonfunction, only transient insulin independence, and relevant immunosuppressive side effects were often observed. Indeed, post-transplant reduction of insulin requirements and improvement in glycemic control rarely lasted long term, with only 10% of islet recipients maintaining insulin independence at 1 year (Bretzel et al., 1999).

A main obstacle in achieving consistent positive results was the diabetogenic effect of corticosteroids and CNIs on β-cell function and survival, as well as on the development of peripheral insulin resistance. Post-transplantation DM occurs in more than 50% of solid organ transplant recipients, including pancreas, with incidence increasing with dose and duration of immunosuppressive therapy. Moreover, drug-dependent increment of lipids is associated with increased allograft loss and toxicity. Glucolipotoxicity may cause β-cell dysfunction and loss (Subramanian and Trence, 2007; Vantyghem et al., 2007; Poitout and Robertson, 2008).

Corticosteroids (dose > 5 mg/day) can induce hyperglycemia by decreasing insulin-mediated glucose uptake in peripheral tissues, with insulin resistance, and by inhibiting insulin production and secretion, with β-cell dysfunction and possibly apoptosis. Increased hepatic gluconeogenesis, reduced glycogen synthesis, and lipolysis also occur. Hyperlipidemia is due to increased VLDL synthesis and down-regulation of LDL receptor and lipoprotein lipase activity, resulting in increased LDL cholesterol and triglycerides and reduced HDL cholesterol. Both metabolic alterations may result in overall increased cardiovascular risk after transplant (Poitout and Robertson, 2008).

CNIs frequently cause hyperglycemia and hyperlipidemia. High-dose tacrolimus (trough levels > 6 ng/ml) is more diabetogenic but less deleterious for lipids than cyclosporine A (trough levels > 300 ng/ml). Hyperglycemia is consequent to decreased insulin synthesis and secretion. Morphological anomalies are present, including reduced β-cell density, loss of secretory granules, cytoplasmatic swelling and vacuolization, and possibly apoptosis. Such alterations seem to be dose-dependent and reversible by drug discontinuation, with no chronic cumulative toxicity on β cells. Effects on insulin sensitivity are still being debated, with some animal and clinical studies reporting increased hyperinsulinemia and insulin
resistance. Dyslipidemia, with increased LDL cholesterol and impaired VLDL and LDL clearance, also occurs. Increased LDL oxidation and lipoprotein levels with accelerated atherosclerosis, as well as increased vascular tone and resistance with hypertension, contribute to a greater cardiovascular risk (Vantyghem et al., 2007).

2.2.2.2 Current Protocols

In late 1990s, new immunosuppressants, such as mTOR inhibitors, sirolimus and everolimus, and novel anti-inflammatory agents, such as TNFα blockers infliximab (chimeric monoclonal antibody) and etanercept (recombinant fusion protein), allowed avoidance of corticosteroids and reduction of tacrolimus dosage in specifically designed ITA protocols (Table 2.1) (Mineo et al., 2008c).

In 2000, the Edmonton group reported remarkable results from a steroid-free protocol including daclizumab at induction and high-dose sirolimus (trough levels 12–15 ng/ml during the first trimester and then 10–12 ng/ml) plus low-dose tacrolimus (trough levels 3–6 ng/ml) at maintenance. After 1 year, virtually all recipients were insulin-free, with normalized HbA1c and absence of severe hypoglycemia. Insulin independence was obtained infusing more than 10,000 IEQ/kg or more than 700,000 IEQ total (full islet mass), from two or more fresh islet transplant infusions (Shapiro et al., 2000; Ryan et al., 2002).

Subsequently, the Miami group successfully introduced a 2-day culture stage in supplemented medium prior to transplant, to allow β-cell recovery from the isolation process, thus increasing islet viability while preserving islet mass. This time period permits the administration of induction strategies that can prevent acute rejection episodes and improve long-term outcomes. It also allows the shipment of islet products to remote facilities for transplantation. The same group also attempted to achieve recipient hematopoietic chimerism and islet graft tolerance infusing HSCs from the same single-donor, without any conditioning, but neither recipient chimerism nor islet graft function persisted after discontinuation of immunosuppression 1 year after transplantation (Froud et al., 2005; Mineo et al., 2008a).

Later on, the Minneapolis group showed that a more potent lymphodepletion at induction, using rabbit ATG (rATG) or a modified humanized OKT3 (hOKT3γ1ala-ala), together with an IT-specific anti-inflammatory strategy using etanercept, achieved insulin independence from a single-donor with less than 10,000 IEQ/kg (marginal islet mass). Sirolimus and low-dose tacrolimus or MMF were used at maintenance (Hering et al., 2004, 2005).

Since the year 2000 many groups have adopted similar immunosuppressive strategies in IT, for a total of more than 700 transplants in about 400 recipients at some 50 centers worldwide, according to data from the Clinical Islet Transplant Registry (CITR), with comparable results in terms of prolonged improvement of glucose metabolism and rate of insulin independence at 1 year, steady at about 70–80% among the most experienced groups (Shapiro et al., 2006, Alejandro et al., 2008).
### Table 2.1 Main clinical islet allotransplantation trials after the year 2000 (adapted from Marzorati et al., 2007)\(^a\)

<table>
<thead>
<tr>
<th>Author</th>
<th>Transplant</th>
<th>T1DM</th>
<th>Number of Pts</th>
<th>IEQ/kg</th>
<th>Induction</th>
<th>Maintenance</th>
<th>Graft function</th>
<th>Graft duration (C-pept)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shapiro et al., 2000</td>
<td>ITA</td>
<td>Yes</td>
<td>7</td>
<td>11,547</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>100% ins ind</td>
<td>&gt;12 months 67%</td>
</tr>
<tr>
<td>Hirshberg et al., 2003</td>
<td>ITA</td>
<td>Yes</td>
<td>6</td>
<td>&gt; 10,000</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>50% ins ind</td>
<td>&gt;22 months 83%</td>
</tr>
<tr>
<td>Hering et al., 2004</td>
<td>ITA</td>
<td>Yes</td>
<td>6</td>
<td>&gt; 10,300</td>
<td>OKT3γ1(Ala-Ala)</td>
<td>Sir, Tac</td>
<td>67% ins ind</td>
<td>&gt;12 months 83%</td>
</tr>
<tr>
<td>Frank et al. 2004</td>
<td>ITA &amp; IAK</td>
<td>Yes</td>
<td>9</td>
<td>15,475</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>100% ins ind</td>
<td>&gt;26 months 57%</td>
</tr>
<tr>
<td>Goss et al., 2004</td>
<td>ITA</td>
<td>Yes</td>
<td>10</td>
<td>&gt;10,000</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>50% ins ind</td>
<td>&gt;26 months 20%</td>
</tr>
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<td>Lehmann et al., 2004</td>
<td>SIK</td>
<td>Yes</td>
<td>9</td>
<td>16,172</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>84% ins ind</td>
<td>&gt;18 months 90%</td>
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<tr>
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<td>ITA</td>
<td>Yes</td>
<td>8</td>
<td>7,271</td>
<td>ATG, Dac, Eta</td>
<td>Sir, Tac later MMF</td>
<td>100% ins ind</td>
<td>&gt;12 months 62%</td>
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<td>ITA</td>
<td>Yes</td>
<td>16</td>
<td>13,552</td>
<td>Dac, Inf</td>
<td>Sir, Tac</td>
<td>100% ins ind</td>
<td>&gt;26 months 80%</td>
</tr>
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<td>Kempf et al., 2005</td>
<td>ITA, SIK, IAK</td>
<td>Yes</td>
<td>22</td>
<td>&gt;10,000</td>
<td>Dac; Bas</td>
<td>Sir, Tac; Eve, CyA</td>
<td>83% ins ind</td>
<td>&gt;12 months 100%</td>
</tr>
<tr>
<td>Ryan et al., 2005</td>
<td>ITA</td>
<td>Yes</td>
<td>65</td>
<td>11,910</td>
<td>Dac, Inf (10); Alem (9)</td>
<td>Sir, Tac</td>
<td>100% ins ind</td>
<td>&gt;60 months 80%</td>
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<td>10</td>
<td>13,806</td>
<td>ATG, then Dac</td>
<td>Sir, Tac, or MMF (2)</td>
<td>100% ins ind</td>
<td>6–21 months 100%</td>
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<td>Toso et al., 2006</td>
<td>IAK</td>
<td>Yes</td>
<td>8</td>
<td>12,530</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>71% ins ind</td>
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<td>6</td>
<td>17,958</td>
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<td>Sir, Tac</td>
<td>50% ins ind</td>
<td>&gt;18 months 83%</td>
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<tr>
<td>Shapiro et al., 2006</td>
<td>ITA</td>
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<td>23</td>
<td>13,473</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>58% ins ind</td>
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<tr>
<td>Ghofaili et al., 2007</td>
<td>ITA</td>
<td>Yes</td>
<td>11</td>
<td>14,312</td>
<td>Dac</td>
<td>Tac, MMFor Sir (1); Exen</td>
<td>73% ins ind</td>
<td>4–30 months 100%</td>
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<td>ITA</td>
<td>Yes</td>
<td>10</td>
<td>11,089</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>80% ins ind</td>
<td>&gt;24 months 80%</td>
</tr>
<tr>
<td>Maffi et al., 2007</td>
<td>ITA</td>
<td>Yes</td>
<td>19</td>
<td>11,477</td>
<td>Dac</td>
<td>Sir, Tac or MMF (6), CyA (1)</td>
<td>65% ins ind</td>
<td>&gt;24 months 33%</td>
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<td>Gillard et al., 2008</td>
<td>ITA</td>
<td>Yes</td>
<td>5</td>
<td>4,700</td>
<td>ATG</td>
<td>Sir</td>
<td>Reduced ins req</td>
<td>&gt;30 months 40%</td>
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<td>Gerber et al., 2008</td>
<td>SIK</td>
<td>Yes</td>
<td>13</td>
<td>345,000 (tot)</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>60% ins ind</td>
<td>&gt;24 months 60%</td>
</tr>
</tbody>
</table>

\(^a\) Data adapted from Marzorati et al., 2007.
<table>
<thead>
<tr>
<th>Author</th>
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<th>T1DM</th>
<th>Number of Pts</th>
<th>IEQ/kg</th>
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<th>Maintainance</th>
<th>Graft function</th>
<th>Graft duration (C-pept)</th>
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<tr>
<td>Cure et al., 2008c</td>
<td>IAK</td>
<td>Yes</td>
<td>7</td>
<td>14,779</td>
<td>Dac, Inf or Eta</td>
<td>Sir, Tac or MMF (2); Aza (1); CyA (2); Pdn (3)</td>
<td>30% ins ind</td>
<td>&gt;36 months 86%</td>
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<td>Gangemi et al., 2008</td>
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<td>Yes</td>
<td>4</td>
<td>24,385</td>
<td>Dac</td>
<td>Sir, Tac</td>
<td>100% ins ind</td>
<td>&gt;30 months 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>11,483</td>
<td>Dac, Eta</td>
<td>Sir, Tac</td>
<td></td>
<td>&gt;21 months 80%</td>
</tr>
<tr>
<td>Mineo et al., 2008</td>
<td>IT+HSC</td>
<td>Yes</td>
<td>6</td>
<td>8,611</td>
<td>Dac, Inf</td>
<td>Sir, Tac</td>
<td>Reduced ins req</td>
<td>&gt;15 months 67%</td>
</tr>
<tr>
<td>Tan et al., 2008</td>
<td>IAK</td>
<td>Yes</td>
<td>7</td>
<td>11,820</td>
<td>Alem</td>
<td>Sir, Tac</td>
<td>60% ins ind</td>
<td>&gt;24 months</td>
</tr>
<tr>
<td>Bellin et al., 2008</td>
<td>ITA</td>
<td>Yes</td>
<td>6</td>
<td>11,872</td>
<td>ATG, Eta</td>
<td>CyA and Eve later MMF</td>
<td>70% ins ind</td>
<td>&gt;36 months</td>
</tr>
<tr>
<td>Froud et al. 2008a</td>
<td>ITA</td>
<td>Yes</td>
<td>3</td>
<td>450,000 (tot)</td>
<td>Alem, Eta</td>
<td>Sir and Tac or MMF</td>
<td>100% ins ind (2)</td>
<td>&gt;24 months 100%</td>
</tr>
</tbody>
</table>

*IEQ/kg = islet equivalent per kilogram; ins ind = insulin independence; ins req = insulin requirement; MMF = mycophenolate mofetil; Pts = Patients; T1DM = type 1 diabetes mellitus; tot = total IEQ. Transplant: IAK = islet after kidney; IT + HSC = hematopoietic stem cells-islet transplant; ITA = islet transplantation alone; SIK = simultaneous islet and kidney transplantation. Induction: Alem = alemtuzumab; ATG = antithymocyte globulin; Bas = basiliximab; Dac = daclizumab; Eta = etanercept; FATG = fresenius antithymocyte globulin; Inf = infliximab; OKT3γ1(Ala-Ala) = humanized anti-CD3 monoclonal antibody. Maintenance: Aza = azathioprine; CyA = cyclosporine A; Eve = everolimus; Exen = exenatide; MMF = mycophenolate mofetil; Pdn = prednisolone; Sir = sirolimus; Tac = tacrolimus. The number of recipients included for a specific variable is shown in parenthesis.
New immunosuppressive or immunomodulatory drugs are being tested to reduce side effects while attempting to use single-donor islet infusion, in order to avoid recipient allosensitization and overcome organ shortage. Preliminary promising results show significant improvements in short-term islet function and survival. The groups in Miami and Edmonton are using alemtuzumab, an anti-CD52 lymphodepleting monoclonal antibody, as an induction agent, while including MMF at maintenance, rather than tacrolimus or sirolimus. Similarly, the group in San Francisco is using rATG and etanercept at induction, with sirolimus plus efalizumab, an anti-LFA1/CD11a leukocyte antiadhesion monoclonal antibody, for maintenance (Froud et al. 2008; Posselt et al., 2008a; Shapiro et al., 2008).

Recently, in order to improve islet function, and possibly survival, as well as to prevent long-term graft exhaustion, the glucagon-like peptide-1 (GLP1) synthetic analogue exenatide, administered subcutaneously at meals, has been given from the time of the first islet infusion (University of Illinois group) or after islet graft dysfunction (Miami and Vancouver groups). The Miami group also reported patients receiving islet retransplants who had been under chronic exenatide treatment prior to the supplemental infusion. Overall, exenatide therapy seems to improve islet engraftment as well as islet graft function and survival, normalizing glucose control and favoring insulin independence (Ghofaili et al., 2007; Faradjì et al., 2008; Froud et al., 2008; Gangemi et al., 2008; Faradjì et al., 2009).

The Edmonton protocol has also been tested in several IAK and SIK trials. In both settings, resulting rates of insulin independence were not always comparable with ITA, varying from 30 to 70% at 1-year post-transplant, but similar stable, normalized glucose control and sustained C-peptide secretion were achieved, also significantly improving function and longevity of kidney grafts without either increasing the risk of kidney rejection or inducing premature decline in its function. Recently, a report from one SIK trial showed successful islet engraftment and function using alemtuzumab and an Edmonton regimen, with 60% insulin independence at 1 year and 100% kidney graft survival for more than 2 years (Toso et al., 2006; Cure et al., 2008a; Gerber et al., 2008; Tan et al., 2008).

2.2.3 Results

2.2.3.1 Clinical Outcomes

Long-term results from different groups have shown that the rate of insulin independence using the Edmonton protocol declines post-transplant to 50% at 2 years, 30% at 3 years, and 10% at 5 years, although 70–80% of recipients have detectable C-peptide (>0.5 ng/ml), with 50–60% reduction in insulin requirement and normalized HbA1c (<6.5%). This progressive islet allograft loss and exhaustion seem mainly due to auto- and allorejection, immunosuppressant-related islet graft toxicity and implantation-site related unsuitability. Recently, the group in Minneapolis reported the achievement of 60% insulin independence for more than 3 years post-transplant, using rATG and etanercept as induction together with mTOR inhibitors plus CNIs (later changed for MMF) at maintenance (Ryan et al., 2005; Bellin et al., 2008).
Significant metabolic improvements are achieved and maintained after IT, even with only partial islet graft function, including stability of glucose control with normalized HbA1c and corrected substrata metabolism, amelioration of insulin sensitivity with reduced insulin requirements, absence of severe hypoglycemia with restored awareness, and improved quality of life. In particular, both the first-phase insulin secretion after an intravenous glucose tolerance test (IVGTT) and the area-under-the-curve (AUC) of C-peptide secretion after an oral mixed-meal tolerance test (MMTT) appear to be restored, with normalization of glucose levels and reduction of glucose excursion at the subcutaneous glucose monitoring system (CGMS) (Figs. 2.2 and 2.3). Notably, glucagon response to hypo- and hyperglycemia appears partially restored, with recovery of sympathoadrenal response and reduced hepatic glucose output, respectively, thus contributing to improved metabolic control after transplant (Luzi et al., 2001; Paty et al., 2002; Rickels et al., 2005a; Meier et al., 2006; Poggioli et al., 2006; Rickels et al., 2006a; Rickels et al., 2007; Gorn et al., 2008; Leitao et al., 2008b; Poggioli et al., 2008; Tharavanij et al., 2008).

Beneficial effects of IT are also evident for long-term diabetic complications, with stabilization or reduced progression of retinopathy and even improvement of neuropathy, with reduced nerve expression of receptor of advanced glycated end-product (RAGE) and increased nerve conduction. The effects on renal function are discordant, with some reports showing a decline in renal function after a long period subsequent to transplantation, whereas others show stability. Acceleration of the diabetic nephropathy as well as renal toxicity per se have been ascribed to immunosuppressive therapy. Prompt implementation of antihypertensive nephroprotective therapies and appropriate recipient selection, especially in ITA, including T1DM patients with virtually normal renal function and presumably slow progression of the diabetic nephropathy, are recommended for limiting post-transplant renal side effects. Results primarily from IAK recipients indicate that IT can induce improvements in cardiovascular and endothelial function (e.g., improved diastolic function, increased nitric oxide production), atherothrombotic profile (e.g., reduced lipid oxidation, delayed intimal media thickening), with fewer cardiovascular events and better survival in IT recipients (90 vs. 50% at 7 years). Overall, together with the improvement in glycemic control, IT seems to be protective for kidney graft function and to increase its longevity. The prolonged C-peptide secretion may contribute to such beneficial effects by reducing nerve dysfunction and increasing blood flow in cardiac and renal districts, with myocardial and glomerular vasodilatation, improving cardiovascular and kidney function, and slowing the progression of diabetic macro- and microangiopathy (Johansson et al., 2000; Wahren et al., 2000; Hansen et al., 2002; Fiorina et al., 2003a, b; Fiorina et al., 2005a, b; Lee et al., 2005; Venturini et al., 2006; Ryan et al., 2006; Del Carro et al., 2007; Fung et al., 2007; Maffi et al., 2007; Senior et al., 2007; Thompson et al., 2008; Warnock et al., 2008, Leitao et al., 2009).

At islet graft dysfunction, long- and short-acting insulin analogues (e.g., glargine and lispro), and/or the incretin-mimetic exenatide, are gradually started. The latter seems to have direct effects on β cells (increased glucose-dependent insulin secretion, restored first-phase secretion, better insulin processing, and higher amylin
Fig. 2.2 Intravenous glucose (IVGTT) (a) and mixed-meal (MMTT) (b and c) tolerance tests, pre- and post-islet allotransplantation. Reproduced with permission from Faradji et al., 2008
Fig. 2.3  Continuous glucose monitoring system (CGMS) profiles pre- (a) and post-islet (b) allotransplantation, and at islet graft dysfunction (c). Different lines represent different days of glucose monitoring. Reproduced with permission from Gorn et al., 2008.
synthesis) and indirect effects on glucose metabolism (reduced glucagon secretion, lower hepatic gluconeogenesis, reduced gastric emptying, and delayed glucose absorption). Whether reduction of apoptosis or regeneration of β cells can occur, as observed in experimental models, is not yet clear. Exenatide may also aid in protecting β cells from immunosuppression-related toxicity. Several side effects (e.g., vomiting, nausea), the risk of pancreatitis, and the possible worsening of preexisting diabetic gastroparesis may limit its use (D’Amico et al., 2005; Ranta et al., 2006; Cure et al., 2008b; Ranganath, 2008).

2.2.3.2 Islet Graft Monitoring

The clinical management of islet transplant recipients relies on the combination of several immune responses and metabolic parameters together with blood trough levels of immunosuppressants and recipient clinical status, including immunosuppressive-related side effects and toxicity symptoms.

The immune alloresponse is monitored principally by mixed lymphocyte alloaction (MLR) and panel reactive alloantibody (PRA) assays for cellular and humoral reactivity, respectively. Evaluation of cytotoxic gene expression levels (e.g., granzyme B) or ATP production in in-vitro stimulated CD4+ T-lymphocytes may represent helpful tools for confirming the clinical picture and the islet graft course, together with cytokine measurement and characterization or other soluble markers. Recurrent autoimmunity can be detected by reappearance of T1DM-specific autoantibodies (e.g., anti-GAD65, anti-IA2, and anti-insulin) and seems to be associated with lower insulin-independence rates and shorter islet graft survival. Histological signs of selective destruction of β-cell allograft as well as autoreactive cytotoxic and memory T cells against specific β-cell epitopes have been also described (Stegall et al., 1996; Bosi et al., 2001; Han et al., 2004; Pinkse et al., 2005; Huurman et al., 2008; Huurman et al., 2009; Mineo et al., 2008b; Monti et al., 2008, Saini et al., 2008).

Monitoring islet graft function for detection or prediction of β-cell dysfunction or failure is based on insulin requirements and blood HbA1c, glucose, C-peptide, and insulin levels measured in the fasting state or after stimulation testing (e.g., intravenous arginine tolerance test, IVGTT, and MMTT). Several indices of islet graft function are derived from these measurements (e.g., acute insulin or C-peptide release, fasting C-peptide/glucose ratio, 90-min glucose). Composite indices are also calculated based on insulin requirements, HbA1c, and the number of infused IEQ, such as the beta score. The use of CGMS or of the MAGE index derived from daily glucose measurements with finger-sticks can help detect early graft dysfunction. Unfortunately, none of these indices is completely reliable or standardized, resulting in detection of metabolic alterations when it is too late to intervene with modifications of the immunosuppressive therapy for rescuing the islet graft (Teuscher et al., 1998; Geiger et al., 2005; Rickels et al., 2005b; Faradji et al., 2007b; Rickels et al., 2007b; Gorn et al., 2008; Baidal et al., 2009).

To date, limited imaging methods are clinically available for visualizing or monitoring the islet graft in vivo. Luciferase-transduced bioluminescence optical
imaging, despite high sensitivity, has limited depth penetration and is not applicable to human studies. High-sensitivity (e.g., 3-tesla) magnetic resonance imaging of islets labeled with different tracers (e.g., superparamagnetic iron nanoparticles) is being tested in animal settings with promising results for clinical application. Positron emission tomography with 18-fluorodeoxy-D-glucose has been used recently in human setting to assess intrahepatic islet engraftment and survival in the immediate postinfusion period. Percutaneous hepatic biopsy is not routinely used owing to procedure-related risks (e.g., bleeding) and lack of certainty of retrieval of islet graft tissue (Eich et al., 2007; Medarova and Moore, 2008).

2.2.4 Complications and Limitations

2.2.4.1 Recipient- and Graft-Related Complications

Acute complications during the islet infusion procedure are rare (<2–6%), and include: intraabdominal bleeding, pleural or abdominal effusions, peripheral portal vein branches thrombosis, and transient transaminitis. Novel radiological techniques, intracatheter-tract coagulants, and recipient peritransplant antithrombotic prophylaxis have reduced their incidence. Intrahepatic focal steatosis and amyloid deposits may follow IT, but their effect on islet graft function and survival is still unclear (Bhargava et al., 2004; Froud et al., 2004; Barshes et al., 2005; Hafiz et al., 2005; Westermark et al., 2008).

The extended period of the islet allograft survival in recent protocols has involved long-term immunosuppression-related side effects in virtually all recipients, primarily common or opportunistic infections (mainly skin, respiratory, and urinary tracts), and direct immunosuppressive toxicity (Table 2.2). Several serious adverse events have been observed that required hospitalization and specific therapy (e.g., profound neutropenia, pneumonia, ovarian cysts), but only one death could be attributed to immunosuppression (viral meningitis). Extremely rare are viral reactivations (e.g., EBV, CMV) or de novo malignancies, with only 13 neoplasms reported (two papillary thyroid carcinomas, six squamous and two basal-cell skin carcinomas, one ovarian and one breast cancer, one pulmonary nodule) in approximately 400 IT recipients according to data of CITR (Cure et al., 2004; Hafiz et al., 2004; 2005; Faradji et al., 2007a, Alejandro et al., 2008; Cure et al., 2008c).

Sirolimus has opposing effects on insulin secretion and action, which appear to be cell-, species-, and dose-dependent, and act by inhibition of insulin-receptor signal transduction and of the kinases regulating the β-cell cycle. Beta-cell dysfunction and reduction of insulin secretion seem to occur only at doses higher than those used in the clinical setting, whereas increased basal and glucose-stimulated insulin levels with reduced apoptosis have been seen at therapeutic concentrations. In skeletal muscle and adipose cells, long-term exposure seems to reduce insulin-dependent glucose uptake and insulin sensitivity, whereas in the short term opposite effects have been observed. Reversible, dose-dependent dyslipidemia also occurs (Subramanian and Trence, 2007; Vantyghem et al., 2007).
Table 2.2  Immunosuppression-related side effects in islet allotransplantation (adapted from Leitao et al., 2008a) 

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>Agent</th>
<th>Treatment</th>
<th>Commentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral ulcers (&gt;70%)</td>
<td>Sirolimus</td>
<td>Topical symptomatic</td>
<td></td>
</tr>
<tr>
<td>Diarrhea (&gt;60%)</td>
<td>Sirolimus</td>
<td>Switch to MMF</td>
<td>Exclude infections (Clostridium difficile/CMV)</td>
</tr>
<tr>
<td></td>
<td>MMF</td>
<td>Switch to enteric-coated MS</td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting (~50%)</td>
<td>Sirolimus/tacrolimus</td>
<td>Symptomatic</td>
<td>Exclude drug toxicity</td>
</tr>
<tr>
<td>Anemia (&gt;90%)</td>
<td>Sirolimus/tacrolimus/ MMF or iron deficiency</td>
<td>Iron supplement, recombinant eritropoetin</td>
<td></td>
</tr>
<tr>
<td>Mild leucopenia (~100%)</td>
<td>Sirolimus/MMF</td>
<td>—</td>
<td>Normalizing within 3 months</td>
</tr>
<tr>
<td>Neutropenia &lt;500/μl (&gt;20%)</td>
<td>Sirolimus/MMF/ Cotrimazol/valganciclovir/ CMV infection</td>
<td>GCSF if &lt;500/μl or &lt;1000/μl with fever</td>
<td>&lt;500/μl with fever (rare, 1/26 recipients) requires hospitalization and broad-spectrum antibiotic therapy</td>
</tr>
<tr>
<td>Severe lymphopenia (&gt;10%)</td>
<td>Alemtuzumab and thymoglobulin</td>
<td>Frequent monitoring for infections</td>
<td>Desirable effect, can last up to one year</td>
</tr>
<tr>
<td>Mild thrombocytopenia (&gt;60%)</td>
<td>Sirolimus</td>
<td>—</td>
<td>Spontaneous remission. ITP in alemtuzumab users - no cases in islet transplant recipients</td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acne (&gt;50%)</td>
<td>Sirolimus</td>
<td>Topical treatment</td>
<td></td>
</tr>
<tr>
<td>Folliculitis (&gt;20%)</td>
<td>Sirolimus</td>
<td>Topical treatment</td>
<td></td>
</tr>
<tr>
<td>Eczema (&gt;10%)</td>
<td>Tacrolimus</td>
<td>Topical treatment</td>
<td></td>
</tr>
<tr>
<td>Metabolic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia (&gt;50%)</td>
<td>Sirolimus</td>
<td>Statins</td>
<td>Increase in LDL-cholesterol</td>
</tr>
<tr>
<td>Impaired insulin secretion</td>
<td>Tacrolimus/sirolimus</td>
<td>—</td>
<td>See immunosuppressive beta cell toxicity</td>
</tr>
<tr>
<td>(variable)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophosphatemia (&gt;90%)</td>
<td>Sirolimus/tacrolimus/MMF</td>
<td>Oral replacement.</td>
<td>Mild</td>
</tr>
<tr>
<td>Hypomagnesemia (&gt;60%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side-effect</td>
<td>Agent</td>
<td>Treatment</td>
<td>Commentary</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>Gonadal</td>
<td>Ovarian cysts (&gt;60%)</td>
<td>Sirolimus</td>
<td>Hormonal Surgery in selected cases</td>
</tr>
<tr>
<td></td>
<td>Altered menses (&gt;60%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Transient hypotension (rare)</td>
<td>Alemtuzumab</td>
<td>Symptomatic</td>
</tr>
<tr>
<td></td>
<td>Increase in blood pressure (&gt;30%)</td>
<td>Tacrolimus/sirolimus</td>
<td>Initiation/increase in antihypertensive medication</td>
</tr>
<tr>
<td></td>
<td>Peripheral edema (&gt;50%)</td>
<td>Sirolimus</td>
<td>Diuretics</td>
</tr>
<tr>
<td>Renal</td>
<td>Proteinuria/albuminuria, decrease in glomerular filtration rate (variable)</td>
<td>Tacrolimus/sirolimus</td>
<td>ACEi/ARB, statins</td>
</tr>
<tr>
<td>Neurological</td>
<td>Tremors, paresthesias, headache, mild depression (&gt;10%)</td>
<td>Tacrolimus</td>
<td>Switch to MMF</td>
</tr>
</tbody>
</table>

\[\text{ACEi} = \text{angiotensin-converting-enzyme inhibitors}; \ \text{ARB} = \text{angiotensinogen receptors blockers}; \ \text{CMV} = \text{cytomegalovirus}; \ \text{GCSF} = \text{granulocyte colony-stimulating factor}; \ \text{ITP} = \text{immune thrombocytopenic purpura}; \ \text{LDL} = \text{low-density lipoprotein}; \ \text{MS} = \text{mycophenolate sodium}.\]
MPA may also have a detrimental effect on β cells, by reducing insulin secretion and inducing apoptosis, as well as on peripheral insulin sensitivity, with most of such data coming from experimental settings, whereas lipid metabolism is not affected. The enteric-coated formulation mycophenolate sodium has recently shown better gastrointestinal tolerability and absorption than MMF and is increasingly used to avoid toxicity from other immunosuppressive drugs (Havrdo et al., 2005; Gao et al., 2007; Subramanian and Trence, 2007; Park et al., 2009).

All the immunosuppressive agents can interfere with islet engraftment and β-cell self-renewal. Indeed, sirolimus has antiproliferative and antiangiogenic effects on duct and islet cells that may impair β-cell engraftment and neovascularization as well as viability and regeneration. Tacrolimus and MPA also have negative effects on duct and islet cell proliferation and differentiation, preventing β-cell neogenesis or replication. No negative effects of everolimus, a newly introduced mTOR inhibitor, on glucose metabolism, have yet been reported, although it can induce dyslipidemia (Buscierie et al., 2006; Cantaluppi et al., 2006a, b; Marcelli-Tourvieille et al., 2007; Nir et al., 2007; Zahr et al., 2007).

Renal toxicity is still a major side effect of immunosuppressive therapy. Tacrolimus may cause acute vasomotor vasculopathy with tubular necrosis and/or chronic fibrotic vasculopathy with glomerulosclerosis and interstitial fibrosis. Moreover, sirolimus may induce acute renal dysfunction and/or chronic proteinuria by increasing glomerular permeability and injury or by suppressing the compensatory renal cell proliferation and repair capacity. Their combined use in IT can have synergic negative effects on renal function per se or may cause the progression of diabetic nephropathy, especially in the presence of pretransplant abnormalities (e.g., microalbuminuria, reduced eGFR), whereas the alternative use of MPA-based regimens could prevent renal injury (Rangan, 2006; Williams and Haragsim, 2006).

Supportive therapy is normally used to counteract systemic immunosuppressant-related side effects, such as angiotensin converting enzyme inhibitors (ACEi) or angiotensinogen receptor blockers (ARB), statins or ezetimibe, together with bone marrow stimulants (e.g., granulocyte-colony stimulating factor, erythropoietin), anti-infective prophylaxes, and dietary supplements (e.g., iron). In most cases prompt treatment of complications minimizes recipient morbidity without any sequela (Hafiz et al., 2005; Faradji et al. 2007).

2.2.4.2 Transplant-Related Limitations

Similarly to the pretransplantation period, many factors can contribute to a significant post-transplantation islet loss especially during the early postinfusion phase, reducing the effective number of functioning islets available during the follow-up period. Because of that often a second or third donor islet infusion is required to achieve insulin independence and durable normalization of glucose control in the recipients.

In particular, during islet infusion, an intravascular instant blood-mediated inflammatory reaction (IBMIR) seems responsible for destroying 50–70% of the infused β cells. An upregulation of tissue factor and other molecules on islet
cell surface after the isolation process is capable of triggering innate immunity via activation of coagulation, complement, inflammation, and natural antibodies, destroying the islets. Peritransplant anticoagulant prophylaxis with heparin can counteract this reaction (Moberg et al., 2002; Johansson et al., 2005; Eich et al., 2007).

A progressive intrahepatic islet graft dysfunction and loss also occurs owing to: poor revascularization, chronic hypoxia, absent reinnervations, proinflammatory milieu, drug toxicity, glucolipotoxicity, fat and amyloid deposition, islet functional overload, premature apoptosis, and lack of regeneration. Several ongoing experiments are aimed at identifying alternative and less hostile implantation sites for islet allograft, with the omental pouch, the thymus, or the bone marrow being the most attractive. Recently, islet autotransplantation in the forearm muscle of a child with genetically determined pancreatitis has shown a prolonged (over 2 years) restoration of insulin secretion and normalized glucose control, while requiring minimal exogenous insulin therapy (Desai et al., 2003; Bhargava et al., 2004; Pileggi et al., 2006; Huang et al., 2008; Merani et al., 2008; Rafael et al., 2008; Westermark et al., 2008; Lau and Carlsson, 2009).

Finally, a major concern of IT is the recipient-wide allosensitization from the multiple HLA-mismatched donor infusions performed to achieve insulin independence, hypothetically jeopardizing the chances of receiving future organ transplants (e.g., kidney or pancreas). Pretransplant PRA levels higher than 15–20% and donor-specific antibodies (DSA) seem associated with reduced islet graft survival. Post-transplant positive PRA levels and de novo DSA may occur after drug dose reduction for persistent or serious side effects (e.g., infections) but their impact on islet graft loss is still unclear. Allosensitization seems absent or minimal under the recommended trough levels of immunosuppression, which also seem able to contain low PRA levels (<5–15%), but it occurs constantly when immunosuppression is discontinued, such as after islet graft failure. High PRA levels (>50%) with DSA and cross-reacting non-DSA may persist for a long time. A slower immunosuppressive tapering could minimize or prevent sudden and massive antigen exposition from residual islet graft (Mohanakumar et al., 2006; Rickels et al., 2006b; Campbell et al., 2007a, b; Cardani et al., 2007).

2.2.4.3 Current Challenges and Future Perspectives

Several technical and clinical limitations still persist in IT (Fig. 2.4). Various cytoprotective strategies and agents are currently under investigation to improve organ preservation and islet yield and survival through the isolation process, such as perfluorocarbons in a two-layer method, new lytic enzyme blends or purification methods, and JNK or caspase inhibitors (Kin et al., 2006; Barbaro et al., 2007; Emamaullee et al., 2007; Sabek et al., 2008; Varona-Santos et al., 2008).

New immunological and possibly tolerogenic strategies, including more selective lymphodepleting drugs, costimulatory blockade, and anti-inflammatory agents, are being tested for increasing islet allograft longevity, preventing allorejection and
Fig. 2.4 Main challenges in clinical islet allotransplantation. Reproduced with permission from Mineo et al. 2008c

recurrent autoimmunity, and reducing recipient side effects and islet graft toxicity (Vincenti and Kirk, 2008). The Clinical Islet Transplant Consortium, including centers in North America and Europe, is starting different phase II–III trials using novel agents (e.g., anti-CD20/B-cell-depleting rituximab, anti-CD80/86 costimulatory blockade belatacept, immunomodulatory deoxyspergualin, anti-inflammatory agent lisofylline, and IBMIR-blocker low-molecular-weight dextran) to improve outcomes. Standardized procedures are also used, with the goal of obtaining approval for IT as a standard health-care procedure, thus allowing for insurance reimbursement. Indeed, costs of IT are very high, approximately $250,000 in the first 2 years post-transplantation, and only a few countries (e.g., Canada) have included this procedure as an optional treatment for selected patients with T1DM.

2.2.5 Conclusions

IT as treatment for brittle T1DM has recently achieved successful graft function, with long-term metabolic improvements and minimal procedure-related complications. Unfortunately, islet recovery from isolation and post-transplant graft durability with the current methods and protocols are still unsatisfactory. Several limitations remain, including auto- and alloimmunity, allosensitization, immunosuppressive-related toxicity, and implantation-site unsuitability. In the near future, improvements in both the isolation process and islet cytoprotection, as well as new, less toxic immunological agents together with tolerogenic protocols, and alternative implantation sites, may overcome such challenges (Ricordi, 2003; Ricordi and Strom, 2004; Shapiro, 2008).
2.3 Simultaneous Pancreas–Kidney Transplantation

Simultaneous pancreas–kidney transplantation (SPK) is considered the best treatment option for patients with T1DM and end-stage renal disease (ESRD). The pancreas transplant can restore euglycemia, providing long-term insulin independence; increase patient survival; stabilize or improve diabetic retinopathy and neuropathy; and, in combination with the kidney transplant, eliminate the need for long-term dialysis (Gruessner and Sutherland, 2005; Leichtman et al., 2008).

More potent immunosuppression agents, improvements in surgical techniques, and better understanding of postoperative complications have led to consistent improvement in SPK transplantation results over the past decade. Drainage of the exocrine pancreas and duodenal segment into the bladder (Fig. 2.5) is used largely in respect to enteric drainage for safety reasons and for identifying changes in transplant function by monitoring urine amylase. Ten-year survival rates for patients and pancreas are 84% and 76%, respectively, among the best long-term survival reported in patients with T1DM/ESRD (Burke and Ciancio, 1997; Burke et al., 1998a; Burke et al., 2001; Gruessner and Sutherland, 2005; Leichtman et al., 2008).

Fig. 2.5 Schematic representation of pancreas-kidney transplantation. (a) Bladder-drained transplant. (b) One option for enteric drainage of pancreas graft in pancreas–kidney transplantation

2.3.1 Clinical Protocols

2.3.1.1 Maintenance Immunosuppression

The incidence of acute rejection (AR) in SPK transplantation has been decreasing over the past decade as a result of advances in immunosuppression. The most common agents for maintenance immunosuppression in SPK transplantation presently are tacrolimus and MMF; other drugs such as cyclosporine A, sirolimus, and azathioprine are also used in different combinations. Corticosteroids are still administered, but there is a trend toward steroid-free immunosuppression protocols with the goal of reducing the consequent adverse effects (Burke et al., 1998b; Ciancio
et al., 2000a; Burke et al., 2004b; Gruessner and Sutherland, 2005; Cantarovich and Vistoli, 2009; Mineo et al., 2008c; Singh and Stratta, 2008).

2.3.1.2 Induction Therapy

The recent therapeutic protocols in kidney and kidney–pancreas transplantation attempt to reduce the incidence and severity of AR as well as prevent long-term chronic (vascular) allograft dysfunction (CAD). The methodologies include reduction of CNIs and of their short- and long-term nephrotoxicity, reduction or avoidance of corticosteroids, use of adjunctive maintenance antiproliferative agents (e.g., mTOR inhibitors), and utilization of new agents, such as nonlymphodepleting monoclonal antibodies (daclizumab or basiliximab), or lymphodepleting monoclonal (alemtuzumab) and polyclonal (e.g., rATG) antibodies. The percentage of patients treated with induction therapy has been increasing and was more than 75% in the most recently reported data from the International Pancreas Transplant Registry (IPTR) 2004 (Gruessner and Sutherland, 2005).

Daclizumab

A series of studies has been published analyzing the safety and efficacy of daclizumab as induction therapy in SPK transplant recipients (Bruce et al. 2000; Burke et al., 2001; Lo et al., 2001a, b; Stratta et al., 2001; Burke et al., 2002a, b; Stratta et al., 2002). The results of a multicenter survey using daclizumab as induction therapy showed a low incidence of AR when used in combination with tacrolimus, MMF, and corticosteroids in SPK transplant recipients (Bruce et al., 2001). The survey reported experience with 71 SPK transplant recipients receiving 4–5 daclizumab doses ($n = 45$) or 1–3 doses ($n = 26$). There were no differences in patient and kidney graft survival rates, 98 vs. 96% and 92 vs. 92%, respectively. However, there was a trend toward improved pancreas graft survival rates in the group receiving 4–5 doses, compared with 1–3 doses (96 vs. 85%, $p = 0.07$). Although more patients receiving 1–3 doses had rejection (54%) than patients receiving 4–5 doses (24%), there was no dose–response relationship between the total number of doses or the adjusted total milligram/kilogram dose and time to rejection. All patients with functioning grafts had good renal and pancreatic allograft function at 6 and 12 months. The overall incidence of major infection was 27%, and there were no differences in the incidence of infection between the two groups. No major adverse events were attributed to daclizumab use. In conclusion, excellent short-term outcomes were noted in this retrospective, multicenter survey of initial experience with daclizumab induction in combination with tacrolimus, MMF, and corticosteroids in SPK transplant recipients.

The safety and efficacy of two dosing regimens of daclizumab as an adjunctive immunosuppressive agent versus no antibody induction in SPK transplant recipients receiving tacrolimus and MMF as primary immunosuppression were investigated in a multicenter, open label, comparative trial (Stratta et al., 2002). SPK transplant recipients were randomized to one of three groups: daclizumab 1 mg/kg every
14 days for five doses (Group I), daclizumab 2 mg/kg every 14 days for two doses (Group II), and no antibody induction (Group III). A total of 166 patients were randomized into the three groups [Group I (n = 70), Group II (n = 74), Group III (n = 22)]. At a minimum follow-up of 3 months, patient, kidney and pancreas graft survival rates were similar among the three groups. However, the rates of acute renal allograft rejection were 18% for Group I, 8% for Group II, and 36% for Group III (p < 0.005). The probabilities of either kidney or pancreas allograft rejection were 22% for Group I, 8% for Group II, and 38% for Group III. At 3 months, the actuarial event-free survival (no AR, allograft loss, or death) rates were 67%, 81%, and 50% in Group I, II, and III, respectively. Although the follow-up was short, this study emphasized the important role of induction antibodies in reducing AR.

Daclizumab in Combination with rATG

The use of new immunosuppressive agents continues to be associated with reduced rates of AR episodes in SPK transplant recipients (Burke et al., 2002a, b). Forty-two SPK transplant recipients were included in a prospective, randomized trial in which they received rATG and daclizumab, tacrolimus, and corticosteroids as baseline immunosuppression. They were then randomized to receive either MMF or sirolimus in addition to baseline immunosuppression. Twenty-two patients received MMF and 20 received sirolimus. There were three episodes of AR (7.1%). These were in the MMF group, all in patients who were off either MMF (wound infection, pneumonia) or corticosteroids. Each of these episodes was corticosteroid-resistant, but responsive to antibody therapy (OKT3 or rATG). Actuarial patient, kidney, and pancreas allograft survivals were 100%, 100%, and 95% in the sirolimus group and 100%, 100%, and 100% in the MMF group (Burke et al., 2002b).

A similar study (Gallon et al., 2007) reported the effect of two tacrolimus-based maintenance regimens on long-term renal allograft function in SPK transplant recipients [tacrolimus/MMF (n = 22) vs. tacrolimus/sirolimus (n = 20)] (Schaapherder et al., 1993). All patients received rATG as induction therapy. The difference from the previous study (Burke et al., 2002b) was that both regimens included prednisone-free maintenance. Patient and pancreas graft survival rates at 6 years were the same, but kidney allograft survival was higher in the tacrolimus/MMF group (90.7% vs. 70.7%, p = 0.09). The incidence of AR and rate of decline in eGFR were similar in both groups (Gallon et al., 2007).

Alemuozumab

A nonrandomized study of 75 pancreas–kidney and solitary pancreas recipients who received alemuozumab (four doses for induction and twelve doses within the first year) and MMF (≥2 gr/day) for induction and maintenance therapy was reported (Gruessner et al., 2005). Thirty milligrams of alemuozumab was given intravenously intraoperatively for induction as well as for maintenance dosing, the latter doses administered when the absolute lymphocyte count increased to 200/mm³ or more; the maximum number of alemuozumab doses was limited to ten within the first
year. In a 6-month follow-up the results were compared with an historical group of 266 consecutive pancreas recipients using rATG induction and tacrolimus maintenance. Patient survival at 6 months for SPK transplant recipients was 90%; for pancreas-after-kidney (PAK) recipients 91%; and for pancreas transplant alone (PTA) recipients 97% ($p \geq 0.4$).

The patient survival rates were not different between the control group and the three study groups ($p \geq 0.06$). Pancreas graft survival at 6 months in the study group for SPK transplant recipients (vs. historical control) was 81% (vs. 79%; $p \geq 0.66$); for PAK recipients 91% (vs. 85%; $p \geq 0.59$); and for PTA recipients 71% (vs. 84%; $p \geq 0.07$). Kidney graft survival in the historical control versus the study group at 6 months for SPK transplant recipients was 81% vs. 85%; ($p \geq 0.2$). The incidence of a first (reversible) rejection episode at 6 months in the study versus the control group for SPK transplant recipients was 41% (vs. 9%; $p \geq 0.0003$); for PAK recipients 14% (vs. 10%; $p \geq 0.89$); and for PTA recipients 19% (vs. 26%; $p \geq 0.36$). In all three recipient categories the median “modification of renal disease” level at 6 months was higher and the median serum creatinine concentration was lower in the study versus control groups, but the differences did not reach statistical significance. The conclusion was that the combination of alemtuzumab and MMF was associated with an acceptable rejection rate (albeit higher than expected for SPK transplants), and good (graft and native) kidney function; it eliminated undesired CNI- and corticosteroid-related side effects, but a long-term follow-up is warranted.

More recently a single-center nonrandomized retrospective sequential study was reported (Kaufman et al., 2006) comparing the effect of alemtuzumab ($n = 50$) and rATG ($n = 38$) as an induction immunosuppression for recipients of SPK transplant given a prednisone-free maintenance regimen in combination with tacrolimus/sirolimus-based maintenance therapy. The overall 1-year patient and graft survival rates were similar for the two treatment groups. The 1-year actual patient survival rates for recipients who received alemtuzumab and rATG were 96% and 100%, respectively ($p = \text{ns}$); the 1-year actual death-censored kidney graft survival rates were 95% and 97.4%, respectively ($p = \text{ns}$); the 1-year actual death-censored pancreas graft survival rates were 92% and 100%, respectively ($p = \text{ns}$); the 12-month actual rejection rates were 6.1% and 2.6%, respectively ($p = \text{ns}$). At 12 months, the serum creatinine values for the alemtuzumab and rATG group were $1.45 \pm 0.36$ and $1.29 \pm 0.43$, respectively ($p = \text{ns}$). Viral infectious complications were statistically significantly lower in the alemtuzumab group. Despite the study limitation, both alemtuzumab and rATG induction were effective in facilitating a prednisone-free maintenance protocol in SPK transplant recipients.

The use of alemtuzumab as induction therapy in SPK transplant recipients has increased substantially. Lately, the impact of steroid-free maintenance immunosuppression in pancreas transplantation using alemtuzumab as induction therapy has been evaluated in a single-center study (Muthusamy et al., 2008), where 102 pancreas transplantations were performed in 100 patients with tacrolimus and MMF, with no maintenance corticosteroids. With a median follow-up of 17 months, patient, pancreas and kidney graft survival (actuarial) was 97%, 89%, and 94%, respectively. Overall incidence of rejection was 25%. The incidence of CMV
and BKV infections was 6.8% and 3.8%, respectively. This experience suggested that alemtuzumab is safe and effective. Furthermore, steroid-free maintenance was achieved in 83% of the patients with a 25% incidence of rejection.

A cautious tone should be used in the context of corticosteroid-free immunosuppression, since a recent report from the Minnesota group showed that occurrence of AR has a far greater impact on kidney graft survival (15 years actuarial) than the development of new onset DM (NODM) (Matas et al., 2008). This may dampen some of the enthusiasm for steroid-free protocols in which the high rate of AR may well translate into worse long-term graft (and hence patient) survival.

In another study (Clatworthy et al., 2007) alemtuzumab was given subcutaneously in 21 SPK transplant recipients. The rate of AR was 14% at 1 year. This route of administration was recommended because lymphocyte depletion was comparable to that seen in patients receiving intravenous alemtuzumab. Recently, alemtuzumab was compared with rATG (Farney et al. 2008) and basiliximab induction therapy (Magliocca et al., 2008). The use of alemtuzumab for induction therapy after SPK transplants was found to be as safe and effective as rATG and basiliximab. Furthermore, the outcome was not inferior to that of the other two induction therapies. It is important to note that there was a higher incidence of CMV infections in the alemtuzumab group and since then a single dose (rather than two) has been used.

2.3.2 Results

2.3.2.1 Patient and Graft Survival

Long- and short-term patient survival rates have improved steadily over the years. Patient survival rates at 1 year have been higher than 90% since the earliest eras, and are now more than 95% for SPK transplantations performed in 2002/2003. Overall, 5-year survival rates have also improved and are higher than 80%. Survival rates at 10 years are 69% for SPK transplantation. One-year pancreas graft survival rates are 85%, and 1-year kidney graft survival rates are 92%. The 5-year pancreas graft survival reached 69%, and the 5-year kidney graft survival was 77% for the 1998/1999 period. The 10-year pancreas and kidney graft survival rates for the 1992/1993 were 46% and 45%, respectively (Gruessner and Sutherland, 2005). These numbers are similar to those in recent reports (Leichtman et al., 2008). At the University of Miami 10-year survival rates for patients, pancreas, and kidney are 8%, 76%, and 51%, respectively (Burke et al., 2001).

2.3.2.2 Diabetic Nephropathy

The effects of pancreas transplantation on diabetic nephropathy are among the most studied benefits of pancreas transplantation. A pivotal study demonstrated that pancreas transplantation can reverse preexisting histological lesions of diabetic nephropathy in the native kidneys, but reversal requires more than 5 years of normoglycemia (Fiorento et al., 1998). Another study reported on 32 T1DM patients
that were evaluated before and 1 year after successful PTA and compared with 30 matched nontransplanted T1DM patients. Evidence for improvement of renal function after pancreas transplantation was found, documented by the reduction of urinary excretion of protein with stable creatinine concentration and clearances (Coppelli et al., 2005).

2.3.2.3 Diabetic Retinopathy

Retinopathy is the most common microvascular complication of DM. The majority of our patients who undergo SPK transplantation have already developed some degree of retinopathy and most of them have received laser therapy (LT). Patients with advanced retinopathy are less likely to benefit from a SPK transplant.

A prospective study evaluated 33 PTA recipients and 35 patients with T1DM who did not receive PTA. At baseline, 9% of PTA and 6% of non-PTA patients had no diabetic retinopathy, 24% and 29% had nonproliferative diabetic retinopathy (NPDR), and 67% and 66% had laser-treated and/or proliferative retinopathy (LT/PDR), respectively. No new case of diabetic retinopathy (DR) occurred in either group during at least 1 year of follow-up. In the NPDR PTA group, 50% of patients improved by one grading and 50% showed no change. In the LT/PDR, stabilization was observed in 86% of cases but worsening of retinopathy occurred in 14% of patients. In the NPDR non-PTA group, DR improved in 20% of patients, remained unchanged in 10%, and worsened in the remaining 70%. In the LT/PDR non-PTA group, retinopathy did not change in 43% and deteriorated in 57% of patients. Overall, the percentage of patients with improved or stabilized DR was significantly higher in the PTA group (Giannarelli et al., 2006). Another report concluded that advanced DR is present in a high proportion of SPK transplant recipients as a consequence of the duration of T1DM (mean of 24.6 years) and the presence of ESRD. More than 90% of patients have stable DR following transplantation (Pearce et al., 2000).

2.3.2.4 Diabetic Neuropathy

Polyneuropathy is a very common (almost 100%) complication of both T1DM and ESRD, and advanced motor, sensory, and autonomic neuropathies are very frequent in patients undergoing SPK transplantation. Improvement or stabilization of gastric function was observed in 12 out of 23 (52%) SPK transplant recipients versus 5 out of 12 (41.7%) T1DM recipients who underwent kidney transplant alone (KTA) (Hathaway et al., 1994). SPK transplant recipients also demonstrated significant improvement in postural adjustment ratio (Navarro et al., 1998). Sensory and motor neuropathies, as measured by nerve conduction studies, have also shown improvement in SPK transplant recipients (Muller-Felber et al. 1993; Navarro et al., 1998).
2.3.2.5 Quality of Life

Patients who received SPK transplants consistently reported an improvement in their quality of life (Sureshkumar et al., 2005). SPK transplantation had a significant positive effect on DM-related quality of life even though SPK transplantation is a complex surgical procedure.

SPK transplantation has been viewed as a higher-cost and higher-risk surgical procedure than kidney transplant, and it is unclear if SPK transplantation offers better health and quality of life outcomes than insulin therapy plus KTA. A study found that both SPK and KTA recipients report better health and quality of life, but SPK transplant recipients also report greater improvement in physical health and in areas that are DM-specific than those of KTA (Gross et al., 2002).

2.3.3 Complications and Limitations

Surgical complications are more common after pancreas transplantation, compared to kidney transplantation. Nonimmunological complications of pancreas transplantation (including thrombosis and graft pancreatitis) account for graft losses in 5–10% of cases. These usually occur within 6 months of transplantation and are as important an etiology of pancreas graft loss in SPK transplantation as AR (Ciancio et al. 1996b; Gruessner et al., 1996; Gruessner and Sutherland, 2001; Gruessner and Sutherland, 2005).

2.3.3.1 Hypercoagulation in SPK

T1DM has been shown to result in hypercoagulation, as assessed by numerous studies involving different components of the clotting cascade. In addition, hyperlipidemia, commonly associated with DM, further contributes to such hypercoagulation. Subsequent to SPK transplantation, with restoration of euglycemia, the lipid profile usually normalizes (Burke et al., 1998) or is treated, when necessary, with lipid-lowering medications. Although the uremic effect on platelets could offset the hypercoagulation associated with DM, our experience suggests that it does not.

In our program, a thromboelastogram (TEG) performed at the time of transplantation surgery has confirmed this hypercoagulable state in a remarkably consistent pattern (Burke et al., 2004). Generally, rheologic assessment, including the combination of shortened prothrombin time (PT), partial thromboplastin time (PTT), and elevated platelet count (>400,000/mm³), fibrinogen (>400 mg/dl), and hematocrit (>40%), along with hyperlipidemia, are all features associated with hypercoagulability and conceptually integrated into the TEG. As each of these factors can vary over time, performing TEG at the time of surgery is the most helpful test in determining the degree of hypercoagulability at transplant.

The pancreas transplant portion of SPK transplant has historically been more prone to thrombosis than other solid organ transplants. This has been ascribed to
several factors, including: the degree of organ injury (i.e., the cumulative effect of preterminal donor injury with hypotension and hypoperfusion) and ischemia–reperfusion damage (Coppelli et al., 2005), which affect all solid organ transplants; technical issues (e.g., the size of the vessels or the method of vascular reconstruction) (Troppmann et al., 1996) [however, others have described low rates of pancreas transplant thrombosis with similar vascular techniques (Sollinger, 1996)]; and the use of desmopressin in the therapy of diabetes insipidus in the donor. This was associated with impaired microcirculation and subsequent pancreas transplant thrombosis (Burke et al., 2004).

When viewed in the context of Virchow’s triad, thrombosis can in fact be predicted. Virchow’s triad incorporates hypercoagulability, endothelial damage, and venous stasis as the criteria for venous thrombosis, and all three criteria are met in solid organ pancreas transplant. The hypercoagulable state is clearly defined in these patients with T1DM and ESRD by the TEG (Burke et al. 2004). The endothelial damage is associated with all solid organ transplants undergoing a period of cold and warm ischemia, with subsequent reperfusion injury after release of the vascular clamps. Such events result in the well-described ischemia–reperfusion injury with endothelial damage from numerous mediators, including cytokines, O$_2$ radicals, and nitric oxide. The venous stasis occurs when the spleen is removed from the tail of the pancreas and the major source of blood flow through the splenic vein is lost. The splenic vein remains with its high capacitance, but only with the limited flow from the arterial side of the pancreas. The superior mesenteric vein similarly no longer receives venous return from the small bowel, and is limited to small pancreatic venous radicals to maintain flow. Furthermore, immunosuppression itself, mostly CNIs (tacrolimus, cyclosporine A), can induce endothelial damage and hypercoagulability by enhancing secretion of procoagulant factors, for example, endothelin (Burke et al., 1999). Thus, pancreas transplant fulfills Virchow’s triad for propensity to venous thrombosis.

The TEG-demonstrated hypercoagulability of patients with DM and ESRD has led us to use heparin intraoperatively when the degree of hypercoagulability is matched with the degree of operative field hemostasis. The PT loss rate of 1% from thrombosis shows that this has been an effective strategy, while reoperating from bleeding is also low (2%) (Burke et al. 2004). Anticoagulation may also confer protection to the distal extremity, which suffers an ischemia–reperfusion injury itself (typically subclinical) after cross-clamping the iliac artery and vein while transplantation is being performed. Since pancreas transplantation can be performed without anticoagulation with a similarly low rate of thrombosis, the demonstration of the hypercoagulable state in T1DM with ESRD by TEG may be more important as a risk factor for atherosclerosis (i.e., an issue for long-term patient survival). When seen in the context of other risk factors for atherosclerosis (e.g., hypertension, obesity, DM, insulin resistance, dyslipidemia, all components of the metabolic syndrome), the greatest benefit of the demonstration of the hypercoagulable state may lie in its subsequent therapy. Appropriate anticoagulation with aspirin therapy or other medications, in addition to the correction of DM, hypertension, renal failure, and dyslipidemia related to SPK transplantation, may result in reduced atherosclerosis.
Although SPK transplantation prolongs patient survival (Burke et al., 2001), recognition and treatment of the hypercoagulable state, along with new approaches to inflammation and atherosclerosis, may allow further improvement.

### 2.3.3.2 Bladder-Drained Pancreas Transplant

Bladder-drained pancreas transplants (Fig. 2.5) are associated with multiple urological (Ciancio et al., 1995; Ciancio et al., 1996b) and metabolic complications, requiring enteric conversion in 14–50% of most reported series, although only in 8% of our 390 consecutive SPK transplants.

Hematuria occurs frequently but generally resolves early after transplantation with conservative measures. Late-occurring hematuria may be caused by formation of a bladder stone on the staple or suture line. Approximately 5% of patients will require interventions such as Foley catheter placement, irrigation, and cystoscopy for evacuation of clots. Urinary tract infections are common; they occur in as many as half of all cases. Although the urinary pH is generally alkaline and maintains pancreatic proenzymes in an inactivated state, a urinary tract infection can reduce the pH enough to activate these digestive enzymes. Enterokinase present in the brush border of the duodenal mucosa may activate the proenzyme trypsinogen and thereby initiate the pancreatic enzyme activation cascade. Other proteases, such as plasmin, thrombin, and fibrolysin, as well as bacterial enzymes, may also activate trypsinogen to trypsin. The severe burning from urethritis is attributed to autodigestion by the activated pancreatic enzymes trypsinogen and chymotrypsinogen. If untreated, these symptoms may progress to urethral disruption and later stricture. Treatment of urethral complications requires both enteric conversion and urological expertise (Ciancio et al. 1996a). Fortunately, this complication has become less common with the use of small duodenal segments.

Metabolic acidosis is caused by the excretion into the bladder of large quantities of alkaline pancreatic secretions. Most patients take supplemental sodium bicarbonate orally to minimize the degree of acidosis. With time, most of these patients are able to decrease their oral sodium bicarbonate intake.

Fluid management can become problematic for these patients because of the potential for relatively large volume losses. Patients are at risk for dehydration, which can be exacerbated by poor intake as a result of gastric-motility problems commonly associated with T1DM. The symptoms from dehydration can be further compounded when patients have preexisting orthostatic hypotension related to diabetic autonomic neuropathy. Fluid balance can be improved in some patients by the administration of fludrocortisone acetate. Out of our 390 patients who underwent SPK transplantation with bladder drainage, 20% were readmitted within the first year for correction of acidosis and/or dehydration. Their serum creatinine concentrations usually returned to baseline after the administration of intravenous fluids with bicarbonate. Occasionally, patients experience a persistent rise in creatinine associated with episodes of dehydration and require conversion to enteric drainage of the pancreatic secretions.
Urine leaks owing to breakdown of the duodenal segment can occur years after transplantation, but are usually encountered within the first 2–3 postoperative months. The causes of early urine leaks are most often technical in nature and generally require surgical correction with prolonged Foley catheter drainage. Late-occurring leaks can be caused by high pressure in the duodenum during urination. The onset of abdominal pain with elevated serum amylase, which can mimic reflux pancreatitis or AR, is a typical presentation. Imaging studies utilizing a cystogram or CT scanning may be necessary to confirm the diagnosis. Operative intervention may be required, including reanastomosis to the bladder or to bowel.

Despite these complications, bladder drainage of the pancreas graft has many advantages. Early and late complications may cause morbidity; however, these are rarely lethal because enteroenterostomy and, hence, potential intraperitoneal enteral spillage can be avoided.

Another advantage of bladder drainage is the ability to monitor the patient for graft rejection. A decrease of more than 50% in urine amylase activity after pancreas transplantation signals possible AR. The decrease in urinary amylase may be the only clinical indication of a problem, with no change in the serum concentration of creatinine or glucose or the activity of serum amylase or lipase. A biopsy of the pancreas should be performed to confirm the diagnosis of rejection.

After the administration of rejection therapy with corticosteroids or antilymphocyte preparations, the need for repeat pancreatic biopsy can be determined by measuring urine amylase activity. If low urine amylase activity persists after rejection therapy, pancreas biopsy is indicated. In contrast, if urine amylase activity is restored to baseline and the blood-glucose concentration remains high after therapy, the causative factor is steroid therapy with insulin resistance, rather than rejection, and pancreatic biopsy is not necessary.

2.3.3.3 Enteric-Drained Pancreas Transplant

When pancreas transplantation was first performed in the early 1970s, the results of enteric drainage (ED) were poor. The small-bowel drainage procedure fell into disfavor because of anastomotic leaks with abscess formation. Resultant sepsis caused high rates of morbidity and mortality. Recently, more centers are experiencing success with ED (Reddy et al., 1999; Stratta et al., 2000; Monroy-Cuadros et al., 2006; Lipshtultz and Wilkinson, 2007) because of improvement in donor management, optimized surgical techniques during organ procurement, better preservation solutions, improvement of the implantation procedure, and new immunosuppressive drugs (Ciancio et al., 1997; 1998; Ciancio et al., 1999; Ciancio et al., 2000a; Gruessner and Sutherland, 2005). Enteric drainage techniques (Fig. 2.5) vary in bowel arrangement, level of anastomosis, site of the recipient small bowel, and choice of either stapled or hand-sewn anastomosis (Di Carlo et al., 1998).

Of the pancreas transplantations performed in 2002/2003, 82% in the SPK, 72% in the PAK, and 57% in the PTA categories were ED. Of the few ED transplants carried out before 1996/1997, most were done with a Roux-en-Y limb of the recipient bowel, but in the 2002/2003 ED pancreas transplantations only 29% used a Roux-en-Y limb (Gruessner and Sutherland, 2005).
The most serious complication of enteric-drained pancreas transplantation is a leak from the anastomotic site. This serious problem occurs 1–6 months after transplantation and results in fever, abdominal discomfort, and leukocytosis. CT scans are helpful in diagnosing the problem. The mandatory treatment is surgical exploration and repair of the enteric leak. Gastrointestinal bleeding may occur at the duodenal–enteric suture line as a result of perioperative anticoagulation and inadequate homeostasis. When conservative management is not sufficient, reoperation is necessary (Reddy et al., 1999; Stratta et al., 2000).

Enteric drainage has some advantages over bladder drainage. First, because exocrine pancreas secretions are enterically directed, metabolic acidosis and dehydration do not occur and bicarbonate supplementation is not needed. Second, this procedure is not associated with urological complications, such as urinary infections, hematuria, bladder stones, and urinary leaks. Third, fewer laboratory tests are required because there is no reason to monitor urinary activity. However, rejection episodes may progress undiagnosed before treatment is started, and this delay increases the possibility of allograft loss.

2.3.4 Conclusions

The optimal treatment for T1DM in the context of ESRD, where the primary goal is to restore normal glucose metabolism and then kidney function, is achieved by whole pancreas and kidney allograft transplantation. The administration of lymphodepleting agents continues to increase, whereas use of IL2 receptor antagonists is declining. The main goal of induction therapy is to provide a strong and long-term immunosuppressive effect for protocols that include steroid avoidance, CNI minimization, or even monotherapy maintenance. The current trend is to reduce or minimize the number of immunosuppressive drugs in order to prevent or avoid side effects and adverse events. The challenge is to find the balance between benefit (protection from AR and long-term graft function) and risk (side effects, infection, cancers).

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For further information including transplant data and annual reports

U.S. Department of Health and Human Services (http://www.hhs.gov); Organ Procurement and Transplantation Network (http://www.optn.org); Scientific Registry of Transplant Recipients (http://www.ustransplant.org); Health Resources and Services Administration (http://www.hrsa.gov); Collaborative Islet Transplant Registry (http://www.citregistry.org); and Clinical Islet Transplant consortium (www.citisletstudy.org).

References


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