Transplant International

Transplant International ISSN 0934-0874

REVIEW

Minimization and withdrawal of steroids in pancreas and islet transplantation

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Keywords
gluco-corticosteroids, immunosuppression, islet transplantation, pancreas transplantation, side-effects, toxicity.

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Received: 29 May 2008
Revision requested: 24 June 2008
Accepted: 14 August 2008

doi:10.1111/j.1432-2277.2008.00761.x

Summary

For reducing the corticosteroid (CS)-related side-effects, especially cardiovascular events, CS-sparing protocols have become increasingly common in pancreas transplantation (PT). Lympho-depleting induction antibodies, such as rabbit anti-thymocyte globulin (rATG) or alemtuzumab, have been widely used in successful trials. The results of various CS-sparing protocols combining calcineurin inhibitors (CNI) and mycophenolate or sirolimus, have been mixed for rejection and survival rates. Most of the studies were uncontrolled trials of low-risk patients, therefore the grade of evidence is limited. Large-scale prospective studies with long-term follow up are necessary to assess risks and benefits of CS-sparing regimens in PT before recommending such strategies as standard practice. Islet allo-transplantation for patients with brittle type 1 diabetes mellitus, less invasive and safer procedure than PT, has been attempted since late 1980s, but diabetogenic immunosuppressants at maintenance, mainly CS and high-dose CNI, prevented satisfactory results (10% insulin-independence at 1-year post-transplant). Since 2000, CS-free and CNI-reducing protocols, including more potent induction [daclizumab, OKT3γ1(ala-ala) anti-CD3 antibody, rATG] and maintenance (sirolimus, mycophenolate) agents, have significantly improved short-term outcomes whereas long-term are still inadequate (from 80% to 20% insulin-independence from 1- to 5-year post-transplant). Main limitations are allo- and autoimmunity, immunosuppression-related islet and systemic toxicity and transplant site unsuitability, which tolerogenic protocols and biotechnological solutions may solve.

Introduction

In pancreas transplantation (PT), the high incidence of corticosteroid (CS)-related side-effects, especially cardiovascular events, negatively affect long-term transplant outcomes and has motivated the introduction of CS-sparing immunosuppressive protocols. Novel and more potent monoclonal antibodies at induction and immunosuppressants at maintenance have been successful in PT recipients with low immunological risk. However, CS-withdrawal has been associated with increased risk of acute rejection in other PT cohorts, while the rate of infective complications needs further evaluation.

In islet allo-transplantation (IT), recent CS-free and calcineurin inhibitors (CNI)-reducing protocols attempted to avoid the diabetogenic effects of immunosuppression

on insulin production and peripheral action. Significant improvements in islet graft function and survival have been achieved in IT recipients with brittle type 1 diabetes mellitus (T1DM) (i.e. poor glycemic control, severe hypoglycemia, progressive complications). However, long-term results are still not satisfactory, and immunosuppression-related toxicity, immune response, and an unsuitable implantation site have to be overcome.

In this review, we describe the main clinical trials that attempted minimization and withdrawal of CS in pancreas- and islet transplant settings, summarizing the results achieved, the complications encountered, and the major problems identified for resolution, with a more complete list of all the studies and relative characteristics shown in Tables 1–5.

Pancreas transplantation

Cardiovascular morbidity and mortality still significantly limit PT long-term outcomes, the progression of cardiovascular complications in part caused by the recipient's pre-existing diseases, and in part attributable to the negative effects of maintenance immunosuppressants, including CS, on the relative risk factors. Indeed, 5-year graft survival rates of more than 23 000 performed in USA in 1998–2003 are 77% for simultaneous pancreas–kidney (SPK), 57% for pancreas-after-kidney (PAK), and 56% for pancreas alone (PTA) [1,2].

Many studies in liver and kidney transplants attempted to withdraw CS, without increasing the risks of rejection or graft loss, with encouraging results. Subsequently, newly introduced immunosuppressants have enabled the reduction of CS at maintenance in PT. In 2004, 25% of kidney-pancreas recipients were receiving CS-free maintenance, and many studies now include CS-withdrawal, rapid elimination, or avoidance. However, because of the lack of large prospective randomized studies proving the efficacy and safety of CS-sparing protocols and the supposedly higher pancreas immunogenicity with increased risk of rejection, CS is still part of the immunosuppression in most PT protocols [2,3].

CS-withdrawal in stable recipients

According to the positive results of sporadic CS-withdrawal under cyclosporin A (CsA)-based immunosuppression, a larger number of PT patients were withdrawn from CS under Tacrolimus (Tac)-based regimen [4,5] (Table 1).

Corticosteroid was initially withdrawn from selected recipients who had stable graft function, without rejection episodes in a retrospective study, showing successful CS-withdrawal 4–40 months after PT in 46% of stable recipients, divided in 174 SPK, 20 PAK, and 13 PTA under Tac and mycophenolate mofetil (MMF) or azathioprine (Aza) maintenance [6,7]. Despite comparable graft and recipient survival with sustained function in stable, low-risk patients, incidence of rejection was relatively high (65–80%), probably because of the lack of lymphodepleting antibody induction.

Simultaneous infusion of donor bone marrow cells (BMC) increased the chance of CS-withdrawal, with 67% of the recipients who received BMC being CS-free 3 years post-transplant as compared with 45% of those who did not [8].

Withdrawal of CS has been attempted in 12 SPK and two PTA recipients who had significant side-effects, but they were resumed in four patients because of acute rejection or intolerance to full-dose of MMF [9]. Because CS-withdrawal was unsuccessful in both PTA recipients, they were excluded from the following prospective randomized study that evaluated a total of 55 stable PT recipients (29 SPK, 26 PAK) with full doses of immunosuppressants randomized to standard maintenance (Tac, MMF, and CS) or CS-withdrawal from 6 to 36 months after transplantation. After 6 months, no patient death, graft loss or increased incidence of rejection was observed [10].

Scheduled rapid CS elimination

In kidney transplant recipients, rapid CS elimination using antibody induction has been associated with better outcome than late CS-withdrawal [11,12].

Accordingly, rapid CS elimination has been tested in PT recipients to reduce CS-related side-effects while aiming at lower acute and chronic rejection rates (Table 2). A group of 40 SPK recipients who received only 6 days of CS was compared with a historical group that received full CS maintenance [13]. Rabbit anti-thymocyte globulin (rATG), Tac, 6-day CS, and either MMF or sirolimus (SRL) were given to the CS elimination group, while equine anti-thymocyte globulin (eATG) or anti-IL2receptor (anti-IL2R) monoclonal antibodies (daclizumab or basiliximab) together with Tac, MMF and CS were given to the historical group. Rejection-free survival rate at 1-year in the CS elimination group was significantly higher as compared with the historical group (97.5% vs. 80.2%, $P = 0.034$), with the former group showing a greater incidence of leukopenia.

Elimination of CS 1 week post-transplant has also been tested in two studies on a total of 44 SPK transplant recipients, where CS was converted to SRL in addition to Tac and MMF maintenance, after receiving rATG induction [14,15]. While early rejection rates were lower at 6 months (pancreas 2.3% vs. kidney 4.6%), a higher

Table 1. CS-withdrawal from stable pancreas transplantation.

Author	No. patients/tx type	Induction	Maintenance (after CS w/d)	Time of CS w/d	% CS-free	Survival/follow up	Acute rejection	Adverse events/comments
Cantarovich *et al.* [4]	40 SPK	rATG or anti-IL2R	CsA/Aza	45 days	65% at 6 months 63% at 1 year 75% at 2 years	Pt: 97% K: 93% P: 80% at 6 months	K: 35% (first 3 months), 5% (after 3 months) P: 3%	Anemia, leukopenia
Cantarovich *et al.* [20]	50 SPK	rATG versus no rATG	CsA/Aza	45 days	NR	Pt: 96% vs. 92% K: 80% vs. 92% P: 72% vs. 72% at 9–60 months	K: 76% (no induction) 36% (rATG)	CMV: 40% vs. 60% Leukopenia: 12% vs. 48%
Corry *et al.* [8]	151 SPK	BMC versus no BMC	Tac/MMF or Tac/Aza	After 2 years	67% vs. 45%	Pt: 91% vs. 98% K: 87% vs. 86% P: 83% vs. 79% at 3 years	BM: 57% No BM: 79%	CMV: 0.7%
Humar *et al.* [9]	12 SPK 2 PTA	ALG	Tac/MMF or Tac/Aza	7–47 months	83% in SPK 0% in PTA	Pt: 100% K: 92% P: 100% at 5–51 months	K: 14%	Leukopenia: 14% CHL reduction
Jordan *et al.* [6,7]	174 SPK 20 PAK 13 PTA	No	Tac/MMF or Tac/Aza	4–40 months	46%	Pt: 89% K: 85% P: 72% at 6 years	80% (on CS) 65% (off CS)	Better HbA1c in CS w/d
Gruessner *et al.* [10]	29 SPK 26 PAK	rATG	Tac/MMF	6–36 months vs. CS maintenance	97% in w/d group	Pt: 100% K: 100% P: 100% at 6 months	SPK: 0% PAK: 7%	Leukopenia and CHL reduction in CS w/d SPK
Kahl *et al.* [43]	35 SPK	rATG	Tac/MMF	>12 months	69%	Pt: 100% K: 97% P: 89% at 35 months	43%	No difference in HbA1c and lipid profiles
Bechstein *et al.* [31], Malaise *et al.* [29], Saudek *et al.* [30]	205 SPK	rATG	Tac/MMF versus CsA-me/MMF	<6 months	Tac 52% CsA-me 36%	Pt: 95% vs. 97% K: 94% vs. 92% P: 89% vs. 72% at 3 years	Tac: 30% CsA-me: 38%	CMV: 34% UTI: 43% BK: 1%
Mark *et al.* [41]	103 SPK 8 PAK 1 PTA	rATG	Tac/MMF	NR	70%	Pt: 96% K: 95% P: 87% at 1 year	NR	CHL and TG reduction, no difference in HbA1c and serum creatinine
Grochowiecki *et al.* [40]	14 SPK	ATG	Tac/MMF	2–16 months	NR	Pt: 100% K: 100% P: 85% at 1 year	NR	CHL and TG reduction

Tx, transplant; SPK, simultaneous pancreas–kidney transplant; PAK, pancreas-after-kidney transplant; PTA, pancreas transplant alone; rATG, rabbit anti-thymocyte globulin; eATG, equine anti-thymocyte globulin; anti-IL2R, anti-interleukin2 receptor monoclonal antibody; ALG, anti-lymphocyte globulin; BMC, bone marrow cells; CsA, cyclosporin A; CsA-me, cyclosporin A microemulsion; Tac, tacrolimus; CNI, calcineurin inhibitor; SRL, sirolimus; Aza, azathioprine; MMF, mycophenolate mofetil; CS, corticosteroids; w/d, withdrawal; Pt, patient; K, kidney; P, pancreas; CMV, cytomegalovirus; HSV, herpes simplex virus; HHV6, human herpes virus 6; BK, polyomavirus; UTI, urinary tract infection; PTLD, post-transplant lympho-proliferative disorder; CHL, cholesterol; TG, triglyceride; GFR, glomerular filtration rate; NR, not reported.

Table 2. Scheduled rapid CS-withdrawal in pancreas transplantation.

Author	No. patients/tx type	Induction	Maintenance (after CS w/d)	Time of CS w/d	% CS-free	Survival/follow up	Acute rejection	Adverse events/comments
Kaufman *et al.* [13]	40 SPK vs. 86 historical control	rATG versus eATG or anti-IL2R	Tac/MMF or Tac/SRL versus Tac/MMF/CS	6 days	NR	Pt: 100% vs. 97% K: 100% vs. 93% P: 100% vs. 92% at 1 year	MMF: 5% SRL: 0% Control: 20%	Leukopenia, no difference in HbA1c
Freise *et al.* [14,15]	44 SPK	rATG	Tac/MMF/SRL	7 days	NR	Pt: 96% K: 93% P: 89% at 1 year	K: 5% P: 2% at 6 months	CMV: 2% BK: 9% PTLD: 2%
Axelrod *et al.* [44]	100 SPK (CS) vs. 100 SPK (CS-free)	eATG or anti-IL2R versus rATG or alemtuzumab	Tac/MMF or Tac/SRL	CS versus CS-free maintenance	NR	Pt: 96% vs. 96% K: 93% vs. 92% P: 90% vs. 92% at 2 years	14% (CS) 4% (CS-free) at 1 year	CMV: 17% (CS) vs. 9% (CS-free)
Hanaway *et al.* [45]	13 SPK, 6 PAK 1 P after islet Tx	rATG	Tac/MMF	6 days	SPK: 85% PAK: 100%	Pt: 100% K: 100% P: 95% at 7 months	SPK: 8% PAK: 14%	NR
Fridell *et al.* [46]	19 PAK (CS w/d) vs. 10 PAK (CS)	rATG	Tac/SRL	5 days	100%	Pt: 94% K: 94% P: 89% at 1 year	NR	CMV: 5%
Kaufman *et al.* [23]	88 SPK	Alemtuzumab versus rATG	Tac/SRL	3 days	95%	Pt: 91% vs. 92% K: 91% vs. 86% P: 92% vs. 97% at 3 years	8% vs. 5% at 2 years	CMV: 6% vs. 19% BK: 4% vs. 13% PTLD: 0% vs. 3%
Margreiter *et al.* [33]	241 SPK	rATG	Tac/MMF versus Tac/SRL	Short-term	NR	Pt: 98% vs. 98% K: 97% vs. 98%, 87% vs. 81% at 6 months	28% vs. 33%	High GFR in MMF
Aoun *et al.* [21]	24 SPK	rATG	Tac/MMF	4 days	59%	Pt: 100% K: 100% P: 96% at 1 year	K: 4% P: 8% at 6 months	Leukopenia: 42% CMV: 17% BK: 4%
Gallon *et al.* [32]	59 SPK	rATG	Tac/MMF versus Tac/SRL	3 days	MMF: 91% vs. SRL: 92%	Pt: 95% vs. 89% K: 91% vs. 71% P: 100% vs. 100% at 6 years	MMF: 18% SRL: 27%	Leukopenia: 30% vs. 10% CMV: 5% vs. 11% BK: 0% vs. 2% PTLD: 5% vs. 5%
Rajab *et al.* [22]	77 SPK, 19 PAK, 1 PTA vs. 124 historical control	rATG versus anti-IL2R	CsA-me/SRL versus CsA-me/MMF/CS	5 days	NR	Pt: 94% vs. 95% K: 96% vs. 98% P: 95% vs. 88% at 1 year	9% vs. 28%	No difference in glucose, creatinine, weight gain, or lipids
Vessal *et al.* [35]	11 PTA, 6 PAK, 5 SPLK vs. 32 historical control	rATG	Tac/MMF	21 days	59%	Pt: 100% vs. 94% P: 96% vs. 81% at 1 year	27% vs. 38% at 1 year	CMV: 14% vs 25% BK: 9% vs. 19%
Muthusamy *et al.* [47]	70 SPK/PAK/PTA	Alemtuzumab	Tac/MMF	NR	86%	Pt: 96% K: 93% P: 87% at 1–26 months	27%	CMV: 7% BK: 3%
Farney *et al.* [24]	17 SPK, 4 PAK	Alemtuzumab versus rATG	Tac/MMF	6 days	NR	Pt: 100% vs. 100% K: 100% vs. 80% P: 94% vs. 100%, median 6 months	25% vs. 20%	NR

For abbreviations see Table 1.

incidence of infections was seen. Indeed, two early deaths were attributed to uncontrollable sepsis; one cytomegaloviral (CMV) infection, two infections by polyomavirus (BK), and one post-transplant lymphoproliferative disorder (PTLD) were also observed.

CS-avoidance

As even a short-term course (1 year) of CS can induce osteoporosis, cataract, and increase cardiovascular risk [16], a recent study in kidney transplant recipients

Table 3. CS-avoidance in pancreas transplantation.

Author	No. patients/tx type	Induction	Maintenance (after CS w/d)	Time of CS w/d	% CS-free	Survival/follow up	Acute rejection	Adverse events/ comments
Cantarovich *et al.* [18]	28 SPK	rATG	CsA-me/MMF	No CS	89%	Pt: 96% K: 96% P: 75% at 4–24 months	K: 7%	CMV: 29% HSV: 14% PTLD: 4%
Cantarovich *et al.* [19]	25 SPK (CS w/d) vs. 25 SPK (no CS)	rATG	CsA-me/MMF	3 months vs. no CS	78%	Pt: 96% vs. 92% K: 96% vs. 88% P: 76% vs.80% at 3 years	4% (CS w/d) 4% (no CS)	CMV: 4% Higher serum creatinine in no CS at 1 and 2 years

For abbreviations see Table 1.

Table 4. CNI-free or CNI monotherapy with alemtuzumab induction in pancreas transplantation.

Author	No. patients/tx type	Induction	Maintenance (after CS w/d)	Time of CS w/d	Survival/follow up	Acute rejection	Adverse events/comments
Gruessner *et al.* [26]	21 SPK, 23 PAK, 31 PAK vs. 266 historical control	Alemtuzumab plus rATG x1 versus rATG (control)	Alemtuzumab/MMF versus Tac/MMF (control)	No CS	Pt: 90% (SPK), 91% (PAK), 97% (PTA) K: 81% (SPK) P: 81% (SPK), 91% (PAK), 71% (PTA) at 6 months	SPK: 41% vs. 14% PAK: 14% vs. 10% PTA: 19% vs. 26%	CMV : 9% vs. 5% PTLD: 0% vs. 2% Higher GFR at 6 months
Kaufman *et al.* [34]	54 SPK vs. 50 SPK historical control	Alemtuzumab	MMF/SRL versus Tac/SRL (control)	3 days	Pt: 92% vs. 96% K: 90% vs. 94% P: 91% vs. 92% at 1 year	21% vs. 6%	30% of CNI-free converted to Tac during 1 year lower serum creatinine if remain CNI-free
Thai *et al.* [25]	30 SPK 20 PAK 10 PTA	Alemtuzumab	Tac	2 days	Pt: 94% K: 87% P: 89% at 22 months	30%	CMV: 12% HHV6: 2% PTLD: 2% Histoplasmosis: 2% Cryptococcal meningitis: 2%

For abbreviations see Table 1.

compared CS-avoidance to CS-withdrawal at 1 week post-transplant, showing a higher incidence of biopsy-proven acute rejection in the CS-avoidance group [17].

For evaluation of CS-avoidance in SPK transplantation, one trial combined a 10-day course of rATG with cyclosporine microemulsion (CsA-me) and MMF in the absence of CS (Table 3), demonstrating an unexpectedly low incidence of acute rejection (7%) and comparable patient and graft survival rates, but relatively high incidence of infections [18]. A prospective comparison study was later performed by the same group on 50 SPK recipients equally having CS-avoidance or CS-withdrawal after 3 months, in combination with rATG, CsA-me, and MMF [19]. Incidence of acute rejection was 4% in both groups. At 1 year, no statistically significant difference was observed in recipients or kidney and pancreas survivals; moreover, at 1 and 2 years post-transplant, recipients in the CS-avoidance group had significantly higher serum creatinine levels as compared with recipients in the CS-withdrawal group.

CS-withdrawal after lympho-depleting induction

The use of lympho-depleting antibody induction remains higher in PT (80% in 2005) as compared with any other organ transplant setting regardless of the maintenance [1,2]. Alemtuzumab and rATG have been widely used in recent CS-withdrawal or -avoidance protocols, while anti-IL2R antibody induction or even no-drugs regimens are less utilized.

A small prospective randomized study (50 patients) showed significant reduction in acute rejection rate in the SPK recipients treated with rATG induction and CsA/Aza maintenance as compared with recipients receiving no induction and same maintenance (36% vs. 76% at 1 year, $P < 0.01$) [20]; unfortunately the incidence of infections

seemed to be higher and leukopenia more common in the induction group. rATG has also been used with other maintenance combinations with similar lower rejection rates [13,14,21,22].

More recently, a growing trend to use alemtuzumab induction is noted. In one retrospective study, 50 SPK recipients who received alemtuzumab induction were compared to 38 SPK recipients who had rATG instead; both groups received Tac and SRL maintenance. After 3 years, patient and graft survival rates did not significantly differ between groups and rejection rates were nearly equivalent, with viral infections significantly lower in the alemtuzumab group [23]. These two lympho-depleting agents were later compared in a prospective randomized study in kidney and pancreas transplant recipients showing similar safety and efficacy [24].

Because of the perceived potency of alemtuzumab, few trials attempted to further reduce maintenance immunosuppression (Table 4). A single dose of alemtuzumab was used to sustain PT recipients receiving Tac alone maintenance, that included 2 days of CS but no anti-metabolites [25]. Patient and graft survivals were similar to those of other studies using CNI and anti-metabolites maintenance, although higher incidences of acute rejections and infections, including two deaths from sepsis were reported. Later, four doses of alemtuzumab and one dose of rATG were given to SPK and PTA recipients receiving MMF maintenance inclusive of alemtuzumab (max 10 doses in the first year) to maintain lymphocyte count $<200/mm^3$, but without CNI and CS [26]. Despite comparable short-term patient and graft survival rates, incidence of acute rejection was significantly higher in CNI- and CS-free SPK patients; moreover, a trend toward higher estimated glomerular filtration rate (eGFR) after 6 months was noted. When combined with CNI/MMF or CNI/SRL alemtuzumab seems to induce a lower T-cell-mediated rejection rate; however, recent studies suggest that it may not prevent antibody-mediated rejection [27,28].

CS-withdrawal and maintenance therapy

To date, the largest randomized, prospective study comparing Tac and CsA-me maintenance was conducted by the Euro-SPK study group [29–31]. Eleven transplant centers compared the two CNI with rATG induction and MMF maintenance in 205 SPK recipients. The number of patients who successfully withdrew from CS was higher in the Tac group compared with the CsA-me group (52% vs. 36%, respectively). While patient and kidney survival rates after 3 years were similar, pancreas survival was superior in the Tac group (89% vs. 72%, $P = 0.002$), with fewer patients as compared with the CsA-me group developing moderate or severe kidney or pancreas rejection.

A small prospective randomized study compared MMF and SRL in combination with rATG induction and Tac maintenance [13]. Both groups showed excellent patient and graft survival rates along with low rejection rate. Kidney and pancreas allograft function was not significantly different. Incidence of lower gastrointestinal symptoms was higher in the MMF than SRL group, but mean leukocytes count was similarly low in both groups. A study that followed from the same groups showed better kidney graft survival in the Tac/MMF group than in the Tac/SRL group (91% vs. 71%, respectively, $P = 0.09$) [32]. Contrary to the expectation of poor kidney graft function in the Tac/SRL group, the slope of eGFR of the two groups did not show significantly different results, hypothesizing that younger donor kidneys used in PT are less susceptible to the synergistic nephrotoxicity of Tac and SRL. A larger prospective randomized study of 241 PT recipients comparing the same immunosuppression by the Euro-SPK group demonstrated a lower eGFR in the Tac/SRL group as compared with the Tac/MMF group [33]. Fewer severe biopsy-proven rejection episodes were also observed in the Tac/SRL group, while more wound-repairing problems and hyperlipidemia occurred.

Sirolimus has also been used in combination with rATG and CsA-me [22]. While the control group in this study received basiliximab instead of rATG induction, the CsA-me/SRL group showed significantly lower incidence of acute rejection than the CsA-me/MMF/CS group (9% vs. 28% at 1 year, $P < 0.01$).

Calcineurin inhibitor-free maintenance therapy associated with rapid CS elimination has been further evaluated in 54 SPK recipients treated with MMF/SRL and in 50 SPK recipients treated with Tac/SRL; both received alemtuzumab induction. While there was no significant difference in graft survival rates, the incidence of acute rejection was higher in the CNI-free group (21% vs. 6%, $P < 0.05$), with the 29.7% of recipients in CNI-free cohort being converted to Tac during the first year of follow up [34] (Table 4).

CS-withdrawal in solitary pancreas transplantation

Although a previous study failed to successfully withdraw CS from PTA recipients [9], a more recent trial demonstrated similar rejection episodes and graft and recipient survival rates between the CS-withdrawal group and the CS-maintenance group [35]. CS was discontinued 21 days post-transplant in 11 PTA, 6 PAK, and 5 simultaneous deceased-donor pancreas and living-donor kidney transplant recipients with low immunologic risk, using rATG induction and Tac plus MMF maintenance. However,

during the first year post-transplant, CS was resumed in 41% of the patients in the CS-withdrawal group because of acute rejection or intolerance to MMF. This study also demonstrated trends toward lower infections in the CS-withdrawal group (CMV 14% vs. 25% and BK 9% vs. 19%, respectively).

Effects of CS minimization and withdrawal

Post-transplant diabetes mellitus (PTDM) develops in 2–50% of all solid organ transplants, depending on the immunosuppression used [36], with 15% of kidney, liver, heart and lung transplant recipients developing PTDM with the current regimens [37]. Glucose intolerance might occur after PT because of the diabetogenic effects of CS and CNI. One recent study reported 19% of PTDM on 144 PT recipients after 3 years receiving CS/CNI maintenance, whereas another trial on 31 SPK CS-treated recipients showed comparable glucose levels than CS-free patients [38,39]. Low-dose CS maintenance might not impair insulin-mediated glucose disposal, although higher insulin levels are required to maintain glucose tolerance with associated higher triglyceride levels.

Favorable trends on some cardiovascular risk factors, such as blood pressure and total cholesterol levels, were noted in some studies, while others showed a parallel reduction of high-density lipoprotein cholesterol levels after CS-withdrawal in CsA-based kidney and SPK transplant recipients [5,9,10,40,41]. Reduction of CS-related side-effects and cardiovascular events are yet to be demonstrated in larger scale prospective studies.

Conclusions

Several studies have shown that CS can be withdrawn in PT, without apparently increasing the risk of acute rejection, but most of them are uncontrolled trials in low-risk patients with small numbers of participants and relatively short-term follow up. The benefits of CS on long-term graft function have to be weighed against the short and long-term complication of CS use, mainly cardiovascular events and glucose control in diabetic patients [42]. Large-scale prospective randomized studies with long-term follow up are necessary to assess risks and benefits of CS-free immunosuppressive regimens in PT before recommending such strategies as standard practice.

Islet transplantation

Type 1 diabetes mellitus is an autoimmune disease of infants and young adults leading to selective destruction of insulin-producing beta-cells within the pancreatic islets [48]. The consequent insulin deficiency causes hyperglycemia with acute (e.g. ketoacidosis) and chronic complications (e.g. neuropathy, retinopathy, nephropathy), dyslipidemia and accelerated atherosclerosis, with increased cardiovascular morbidity and mortality, and poorer quality of life [49,50].

Exogenous insulin is the standard therapy with tailored diet and physical exercise. Novel insulin formulations and infusion-pump technologies have significantly improved glycemic control while reducing complications [42,50,51]. Unfortunately, 20% of patients can not achieve good and stable metabolic control or avoid hypoglycemia and complications because of the concomitant alteration of the 'contra-regulatory system' [52]. In addition, intensive treatment is associated with severe hypoglycemia episodes and increased cardiovascular events [53–56].

Islet allo-transplantation is an attractive treatment capable of restoring a relatively physiological 'glucose sensing' and insulin secretion in patients with brittle T1DM, and is a lesser invasive procedure with fewer complications, in terms of related morbidity and mortality, when compared with PT. Current indications include patients with negative stimulated C-peptide (≤0.3 ng/ml) and imminent or end-stage renal disease who will receive or already has had a kidney transplant (SIK or IAK), to protect graft's longevity. In addition, IT alone (ITA) transplantation is a valid option for patients with normal or minimally-altered renal function and frequent, acute and severe metabolic complications (life-threatening hypoglycemia, ketoacidosis, and hyperglycemia); and/or incapacitating physical and emotional problems with insulin therapy; and/or failure of insulin management to prevent complications [57–60].

Criteria for multi-organ, deceased donor management and methods for pancreas procurement and preservation have been defined [61–63]. An automated method for mechanically enhanced digestion of the organ, using collagen-lytic enzymes to extract islets, and semi-automated purification techniques using continuous density gradients to divide endocrine from exocrine cells, are used [64–67]. Beta-cell content and function is then assessed to define product's suitability prior to transplant [68]. IT is performed by gravity infusion into the portal vein through percutaneous trans-hepatic approach, under fluoroscopic and ultrasound guidance, with local anesthesia (Fig. 1) [69,70].

Historical protocols

Initial clinical trials of IT in T1DM patients started in late 1980s, mainly as simultaneous islet-kidney transplantation (SIK) and islet-after-kidney transplantation (IAK), or combined with other solid organ transplants (Table 5).

Table 5. Clinical islet allo-transplantation trials (adapted from Marzorati *et al.* [59]).

Author	Transplant	T1DM	Patients no.	IEQ/kg	Induction	Mantainance	Graft function	Graft duration (c-pept)
Mintz *et al.* [151]	IAK	Yes	4	na	None	CsA	na	na
Scharp *et al.* [152]	IAK	Yes	1	na	MALG	CsA	ins ind	22 days
Tzakis *et al.* [71]	LIT	No	9	na	None	Tac	44% ins ind	48–186 days 100%
Scharp *et al.* [153]	ITA	Yes	3	6319	MALG	Pdn, Aza, CsA	Reduced ins req	2 weeks
	IAK		3	6161		Pdn, Aza, CsA		2 weeks–10 months
	IAK		3	13 916		Pdn, Aza ± CsA		>30, >150, >180 days
Ricordi *et al.* [72]	LIA	Yes	10	na	None	Tac, Pdn	100% c-pept	5–19 months (6)
	SIK		9					>19 months (1)
Socci *et al.* [154]	SIK	Yes	2	na	rATG	Pdn, CsA, Aza	Reduced ins req	>3 months (3)
	IAK		4		rATG (2)	Pdn, CsA, Aza		
Gores *et al.* [155]	OLTx & ICTx	No	6	3030	None	Tac, MP	100% ins ind	>8 months
Mazzaferro *et al.* [156]	ILT	No	1	na	None	CsA	ins ind	na
Hering *et al.* [74]	IAK	Yes	1	6140	rATG, Pdn	Pdn	Reduced ins req	>6 months
Ricordi *et al.* [157]	LIA + BMC	No	2	7631; 5851	None	Tac, Pdn	Reduced ins req	na
Lenisa *et al.* [158]	SIK	Yes	7	350 000 (tot)	rATG	CsA, rATG	Reduced ins req	>6 months 68%
	IAK		14					12–48 months 52%
Rilo *et al.* [159]	LIA	Yes	11	na	None	Tac	55% ins ind	2–6 months
	SIK		11			Tac, Pdn	None	54 months
	LIA		4			Tac, Pdn	None	1–49 months
	LIA + BMC		1			Tac, Pdn	None	14 months
	SIK + BMC		6			Tac, Pdn	Reduced ins req	17 months
Ricordi *et al.* [160]	OLTx & ICTx	No	6	3030	None	Tac, Pdn	na	>12 months 67%
Alejandro *et al.* [73]	SIK	Yes	7	9092–21 185	OKT3	MP, Aza,CsA	Reduced ins req	>12 months 80%
	IAK		1					>6 years 25%
Secchi *et al.* [75]	SIK	Yes	8	9433	rATG	Pdn, CsA, Aza	45% ins ind	>4 months (8)
	IAK		13		rATG	Pdn, CsA, Aza	50% ins req	
Tibell *et al.* [76]	SIK	Yes	2	>5700	FATG	CsA, MMF, Pdn	ins ind	6 months
	IAK		1	8800	FATG	CsA, MMF, Pdn		8 weeks
Keymeulen *et al.* [161]	IAK	Yes	7	2100–5300	±rATG	Pdn, CsA, Aza	28% ins ind	>12 months 43%
Bretzel *et al.* [78]	SIK	Yes	12	5414	FATG	Pdn, CsA, Aza	Reduced ins req	>3 months 100%
	IAK		12	8732	FATG	Pdn, CsA ± Aza		>3 months 83%
Oberholzer *et al.* [77]	ILT	Yes	1	5625	rATG	Pdn, CsA, Aza	85% ins ind	3–63 months
	SIK		8	3162–9555	Bas (after	CsA, MMF, Pdn		
	IAK		4	3763–8800	1997)	(after 1998)		
Pattou *et al.* [162]	IAK	Yes	1	10 000	FATG, Pdn	CsA, Pdn, Aza, FATG	Reduced ins req	>1 month
Shapiro *et al.* [83]	ITA	Yes	7	11 547	Dac	Sir, Tac	100% ins ind	>12 months 67%
Tibell *et al.* [163]	SIK	Yes	5	5700–13 500	FATG (2),	CsA, MMF, Pdn	Reduced ins req	>12 months 30%
	IAK		2		Bas (3)	CsA, MMF, Pdn		
					FATG (1),			
					Bas (1)			
Benhamou *et al.* [164]	IAK	Yes	10	9030	Bas, MP	CsA, MMF, Tac (2)	20% ins ind	>10 months 50%
Hirshberg *et al.* [165]	ITA	Yes	6	>10 000	Dac	Sir, Tac	50% ins ind	>22 months 83%
Hering *et al.* [87]	ITA	Yes	6	>10 300	OKT3γ1 (Ala-Ala)	Sir, Tac	67% ins ind	>12 months 83%
Frank *et al.* [166]	ITA	Yes	9	15 475	Dac	Sir, Tac	100% ins ind	>26 months 57%
	IAK		4					>26 months 20%
Goss *et al.* [167]	ITA	Yes	10	>10 000	Dac	Sir, Tac	50% ins ind	>18 months 90%
Lehmann *et al.* [168]	SIK	Yes	9	16 172	Dac	Sir, Tac	84% ins ind	>12 months 89%
Pileggi *et al.* [89]	IT + HSC	Yes	3	8629	Dac	Tac, MMF, MP,	Reduced ins req	pnf, 45 days,
	IT + HSC		2	7981–10 669	rATG	CsA, MMF		>12 months
	SIK		1	2464	Dac	Tac, MMF, MP		130 days (1)
	IAK		7	9092–21 185	OKT3	CsA, Aza, MP		>24 months
								>14 years 25%
Hering *et al.* [88]	ITA	Yes	8	7271	rATG, Dac, Eta	Sir, MMF, Tac	100% ins ind	>12 months 62%
Froud *et al.* [85]	ITA	Yes	16	13 552	Dac, Inf	Sir, Tac	100% ins ind	>26 months 80%
Kempf *et al.* [169]	ITA, SIK, IAK	Yes	22	>10 000	Dac, Bas	Sir,Tac; Eve, CsA	83% ins ind	>12 months 100%

Table 5. continued

Author	Transplant	T1DM	Patients no.	IEQ/kg	Induction	Mantainance	Graft function	Graft duration (c-pept)
Ryan *et al.* [91]	ITA	Yes	65	11 910	Dac, Inf; Alem	Sir, Tac	100% ins ind	>60 months 80%
Warnock *et al.* [170]	ITA	Yes	10	13 806	rATG, Dac	Sir, Tac, MMF	100% ins ind	6–21 months 100%
Toso *et al.* [171]	IAK	Yes	8	12 530	Dac	Sir, Tac	71% ins ind	>12 months
O'Connell *et al.* [172]	ITA	Yes	6	17 958	Dac	Sir, Tac	50% ins ind	>18 months 83%
Shapiro *et al.* [90]	ITA	Yes	23	13 473	Dac	Sir, Tac	58% ins ind	>24 months 70%
Ghofaili *et al.* [119]	ITA	Yes	11	14 312	Dac	Tac, MMF, Sir (1); Exen	73% ins ind	4–30 months 100%
Badet *et al.* [173]	ITA	Yes	10	11 089	Dac	Sir, Tac	80% ins ind	>24 months 80%
Maffi *et al.* [112]	ITA	Yes	19	11 477	Dac	Sir, Tac, MMF (6), CsA	65% ins ind	>24 months 33%
Gillard *et al.* [174]	ITA	Yes	5	4700	rATG	Sir	40% reduced ins req	>30 months 40%
			5	6400		Sir, Tac	60% ins ind	>24 months 60%
Kaplan *et al.* [175]	ITA	Yes	1	450 000 (tot)	Dac, Eta	Tac, MMF, Sir then Dac	ins ind	>20 months
Gerber PA *et al.* [176]	SIK	Yes	13	345 000 (tot)	Dac	Sir, Tac	31% ins ind	>48 months 40%
Cure *et al.* [102]	IAK	Yes	7	14 779	Dac, Inf or Eta	Sir, Tac or MMF (2); Aza (1), CsA (2); Pdn (3)	30% ins ind	>36 months 86%
Gangemi *et al.* [120]	ITA	Yes	4	24 385	Dac	Sir, Tac	100% ins ind	>30 months 50%
			6	11 483	Dac, Eta	Sir, Tac; Exen		>21 months 80%
Mineo *et al.* [89]	IT + HSC	Yes	6	8611	Dac, Inf	Sir, Tac	Reduced ins req	>15 months 67%

T1DM, type 1 diabetes mellitus; IEQ, islet equivalent; NA, not available; Ins Ind, insulin indipendence; Ins Req, insulin requirement; C-pept, c-peptide positivity (>0.5 ng/ml); PNF, prymary nonfunction; Tot, total IEQ; in parentesis the number of recipents; IT + BMC, islet transplant + whole bone marrow cells; IT + HSC, hematopoietic stem cells-islet transplant; ITA, islet transplantation alone; IAK, islet-after-kidney transplantation; ILT, lung-islet transplantation; LIT, liver-islet transplantation; LIA + BMC, liver-islet transplantation + whole bone marrow; SIK, simultaneous islet-kidney transplantation; rATG, rabbit anti-thymocyte globulin; Bas, basiliximab; Alem, alemtuzumab/Campath-1H; Dac, daclizumab; Eta, etanercept; FATG, Fresenius anti-thymocyte globuline; hOKT3γ1 (Ala-Ala), humanized anti-CD3 monoclonal antibody; Inf, infliximab; MALG, Minnesota anti-lymphoblast globulin; CsA, cyclosporin A; Eve, everolimus; MMF, mycophenolate mofetil; MP, methylprednisolone; Pdn, prednisolone; Sir, sirolimus; Tac, tacrolimus; AZA, azathioprine; Exen, exenatide (synthetic analog of the glucagon-like peptide-1).

Immunosuppressive regimens used were those of the kidney transplant setting, based on CS (prednisolone or methylprednisolone), Aza and/or CsA, with polyclonal antibodies (animal-derived ATG) as induction in few trials. Islets were injected into the liver circulation during the main surgery or by transient percutaneous intraportal catheter [57].

The first successes were registered in early 1990s in islet cluster allograft, using the recently introduced Tac, a new CNI with superior immunosuppressive effects and fewer side-effects than CsA [71–73]. In late 1990s, a more selective lympho-depleting induction was attempted using basiliximab, an anti-IL2R chimeric monoclonal antibody, to reduce acute rejection episodes. Similarly, the anti-CD3 OKT3 was tested but soon abandoned because of severe cytokine release. Moreover, a de novo purine synthesis inhibitor MMF, pro-drug of Mycophenolate Acid, became available for maintenance therapy, showing equal immunosuppressive efficacy but lower nephrotoxicity than CNI [74–77].

The overall results of these clinical trials were not satisfactory, with limited islet allograft survival, high rates of primary islet nonfunction and only transient insulin independence, when IT was performed clustered with other allografts under CS/CNI regimens. Despite detectable C-peptide with significant reduction of insulin requirements and improved metabolic control, 30% of the recipients showed graft function after 1 year, but only 10% of them were insulin-free. A main obstacle in achieving better results was the diabetogenic effect of CS and CNI on beta-cell function and on insulin sensitivity, with drug-related increase of lipids levels also associated with allograft injury [78,79].

Corticosteroid induces hyperglycemia mainly by reducing insulin-mediated glucose uptake and utilization in peripheral tissues with insulin resistance, while the issue of direct beta-cells toxicity through inhibition of insulin production and secretion is still controversial, probably depending on dose and time of exposure. Secondary dyslipidemia is characterized by increased total and LDL-cholesterol and triglycerides with reduced HDL-cholesterol [80,81].

Calcineurin inhibitors frequently cause hyperglycemia and hyperlipidemia. High-dose Tac (trough levels > 6 ng/

1	2	3	4	5	6
Donor/Organ	**Pancreas recovery**	**Islet processing**	**Intra-hepatic site**	**Drug toxicity**	**Immunity**
Age/BMI Fibrosis Fat infiltration Brain death Cause of death Intensive care	Recovery technique Warm/cold ischemia Preservation solution Organ shipment	Enzyme Purification Endotoxin Culture Cell-viability Cell-potency	IBMIR Hypoxia Gluco-toxicity Apoptosis Steatosis	Beta-Cell toxicity Lipo-toxicity Anti-proliferation Anti-angiogenesis	IBMIR Allo-rejection Auto-immunity Allo-sensitization

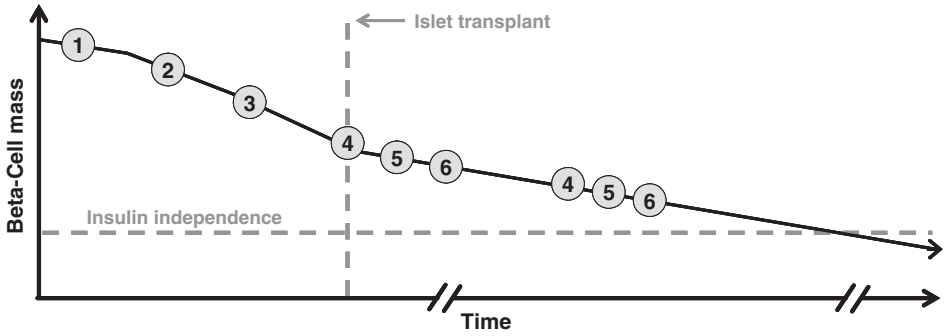

Figure 1 Factors influencing islet allo-transplantation (adapted from Pileggi *et al.* [127]).

ml) is more diabetogenic but less deleterious on lipids metabolism than CsA. Hyperglycemia results from decreased insulin synthesis and secretion, with histologic abnormalities, such as: diminished beta-cells density, loss of secretory granules, cytoplasmatic swelling and vacuolization, and apoptosis. These alterations are dose-dependent and reversible by drug discontinuation, without cumulative toxicity on beta-cells. Effects on insulin sensitivity are still debated, various reports suggesting hyperinsulinemia with insulin resistance. Dyslipidemia and accelerated atherosclerosis together with increased vascular tone and hypertension may also occur [80,82].

Current protocols

In 2000, the introduction of novel immunosuppressive agents, such as the anti-IL2R humanized monoclonal antibody daclizumab at induction, and the mTOR inhibitor SRL at maintenance, has allowed for avoidance of CS and reduction of Tac dose, in specifically-designed ITA protocols (Table 5).

The Edmonton group reported remarkable results using a protocol that included daclizumab induction, with high-dose SRL (trough levels 12–15 ng/ml in the first 3 months, then 10–12 ng/ml) plus low-dose Tac maintenance (trough levels 3–6 ng/ml), using multiple fresh islet infusions. All recipients became insulin-independent, with normalized HbA1c and absence of severe hypoglycemia, 80% of them remaining insulin-free after 1 year [83,84]. Insulin independence was obtained by infusing collectively a minimum of 5–10 000 IEQ/kg (or 350–700 000 IEQ total), usually from multiple (2–4) donors.

The Miami group achieved similar results by culturing the islets for 2 days in supplemented media prior to transplant, to help beta-cells in recovering from the damage of isolation, increasing islet mass and viability, and to allow an appropriate administration of induction drugs. Moreover, the use of anti-inflammatory agents just before the islet transplant, such as the TNF-α blockers infliximab and Etanercept, proved to be limiting peri-infusion inflammation and early beta-cell loss, increasing islet engraftment and survival [85,86]. In addition, the Minnesota group obtained insulin independence from single donor and suboptimal islet mass using the modified anti-CD3 humanized monoclonal antibody OKT3γ1(ala-ala) or rabbit-ATG at induction for lympho-depletion [87,88]. Finally, in a few clinical trials, whole donor bone marrow or hematopoietic stem cells were co-transplanted without ablative conditioning to induce recipient chimerism and/or graft tolerance, but no islet allograft survived the immunosuppression weaning [86,89].

Despite stable, normalized glucose control, prolonged absence of hypoglycemia, reduction of complications, improved cardiovascular function and better quality of life, only 10–20% of recipients remained insulin-free after 5 years, although 80–90% of them exhibited a C-peptide >0.5 ng/ml with 60% reduction in insulin requirement [90–95]. Notably, C-peptide seems to exert beneficial effects on nerve function and blood flow, with myocardial

vasodilatation and reduced glomerular hyperfiltration and albuminuria, thus slowing down the progression of nephropathy [96–98].

Acute complications of islet infusion procedure are rare (<2–6%), including intra-abdominal bleeding or effusions, peripheral portal vein partial thrombosis and catheter obliteration by islets [99]. Advanced radiological and infusion techniques, intra-hepatic catheter-tract coagulants, and peri-procedural anti-thrombotic prophylaxis have reduced their incidence [69,70,99,100].

The sustained islet graft survival of recent protocols has unveiled the occurrence of long-term immunosuppression-related side-effects, including common or opportunistic infections (i.e. urinary and respiratory tracts), or viral re-activation (i.e. EBV, CMV or HSV), all resolved after specific treatments. To date, only seven *de novo* malignancies have been reported in the over 700 recipients of an IT performed using the current protocols [90,99–105].

Sirolimus has contradictory effects on insulin secretion and action. In skeletal muscle and adipose cells, long-term exposure seems to reduce insulin-dependent glucose uptake and insulin sensitivity; in beta-cells, the reduction of insulin secretion seems to arise only at doses higher than the ones used in clinical setting, whereas improved basal and glucose-stimulated insulin secretion, with reduced beta-cells apoptosis, seem to arise at therapeutic concentrations. Reversible dose-dependent dyslipidemia also occur. No negative effects of everolimus, a newly introduced mTOR inhibitor, have been reported on glucose metabolism, while it can induce dyslipidemia [82,106,107].

Mycophenolate mofetil seems to have little detrimental effects on insulin secretion while lipids metabolism is not affected. Recently, the new enteric-coated formulation Mycophenolate Sodium has shown a superior tolerability profile, and is used when toxicity from the other drugs is persistent [82,106,108,109].

Nephrotoxicity can be a side-effect of the combined use of immunosuppressive drugs, especially when previous alterations in renal function are present (e.g. microalbuminuria and reduced eGFR). Tac can induce acute vasomotor vasculopathy and tubulopathy and/or chronic fibrotic vasculopathy and interstitial fibrosis. Similarly, SRL can cause acute renal dysfunction and/or chronic proteinuria [106,107,110–113].

Furthermore, SRL has demonstrated anti-angiogenic properties, and together with Tac and MMF, can have anti-proliferative and anti-differentiating effects on ductal and islet cells, especially at the high concentrations reached in the hepatic circulation, that might impair beta-cells engraftment and revascularization as well as viability and regeneration, preventing both neogenesis and/or self-replication [114–118].

In case of islet graft dysfunction with rising glucose levels, little doses of insulin are required to maintain metabolic stability. Recently, exenatide, a synthetic analog of the glucagon-like peptide-1, has been introduced in addition or even as substitution of insulin therapy. Indeed, it reduces glycemic levels by decreasing glucagon secretion, gastro-intestinal empting and glucose absorption and, at least in experimental models, by improving beta-cells function and survival, with possible cell regeneration [119–121].

During the intra-hepatic islet infusions, an instant blood-mediated inflammatory reaction is responsible for the destruction of 50–70% of the beta-cells, attributable to the up-regulation on islets surface after the isolation mainly of tissue factor that is able to trigger coagulation and inflammation. Anti-coagulants (i.e. heparin) in the transplant media and as peri-transplant recipient prophylaxis may counteract this reaction [122,123].

A concern, whose clinical impact remains to be determined, is the risk of recipient allosensitization, especially when multiple donor infusions are used, and possibly relates to the lack of HLA matching to avoid recurrent autoimmunity. Persistence or recurrence of T1DM-specific autoantibodies has been associated with early islet graft failure [124]. Allo-sensitization is unusual in IT recipients under proper immunosuppression and its influence on islet graft survival remains uncertain, while it generally occurs if complete immunosuppressive drugs discontinuation takes place, as after islet graft failure [125].

Future perspectives

Optimization of strategies to prevent pre- and post-transplant islet loss is currently being tested in different institutions worldwide, and despite many challenges, results continue to constantly improve in clinical and experimental settings (Fig. 1) [126,127].

Ongoing studies aim at identifying alternative, less hostile implantation sites, in combination with biocompatible devices or immuno-protective islet encapsulation [128–130]. New protocols including more potent and selective lympho-depletion, immuno-modulatory and co-stimulatory blockade agents, may increase islet graft survival while avoiding beta-cells toxicity [131–135]. Different strategies have induced hematopoietic chimerism or operational tolerance with acceptance of islet grafts in animals as well as of solid organs in the clinical setting, using minimal irradiative and/or pharmacological nonmyeloablative conditionings followed by donor hematopoietic stem cells infusion [136–140]. Different donor- or recipient-derived cells with immuno-modulatory properties (i.e. lymphocytes, mesenchimal stem cells, regulatory

T-cells, tolerogenic dendritic cells) may augment the chances of long-term graft acceptance [141–143].

Surrogate human- or animal-derived insulin-producing cells are an attractive option to overcome organs shortage, providing a renewable source of beta-cells. Hepatic and pancreatic nonendocrine cells, or adult hematopoietic, mesenchimal, and embryonic stem cells have been manipulated to obtain cells capable of secreting insulin in response to physiological concentration of glucose, as well as xenogeneic islets (i.e. porcine) are being tested, but present results are still far from clinical applicability [144–149].

Conclusions

Islet allo-transplantation for brittle T1DM using current protocols has led to successful engraftment and good short-term graft function. Improvements in isolation and transplant procedures have made IT a feasible and minimally invasive therapeutic approach for selected patients. However, long-term islet graft survival is still low and several obstacles persist, including immunosuppression-related beta-cells and systemic toxicity, allo- and auto-immnune responses and an unfavorable transplantation site. Results are progressively improving and less noxious immunosuppressants or tolerogenic protocols, alternative implantation sites, immuno-protective encapsulation or biocompatible devices, surrogate or xenogeneic insulin-producing cells, together with pancreas and islets processing optimization, will overcome the current challenges [150].

For further information including transplant data and annual reports:

US 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), and the Collaborative Islet Transplant Registry (http://www.citregistry.org).

Supporting

DM is supported by a Post-Doctoral Fellowship in 'Advanced Technologies and Therapies in Surgery', Department of Surgery, Tor Vergata University of Rome.

This work was supported by: National Institutes of Health/National Center for Research Resources (5U42 RR016603, M01RR16587); Juvenile Diabetes Research Foundation International (4-2000-946 and 4-2004-361); National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases (1R01 DK55347, 1R01 DK50759, 1R01 DK056953-02, 1R01 DK025802, 1R01 DK25802-21; 1R01 D59993-04); State of Florida; a contract for support of this research, sponsored by Congressman Bill Young and funded by a special congressional out of the US Navy Bureau of Medicine and Surgery, is currently managed by the Naval Health Research Center, San Diego, CA; and the Diabetes Research Institute Foundation (http://www.diabetesresearch.org).

Acknowledgements

We deeply thank Dr Antonello Pileggi for constructive discussions and reviewing and Mr John Wilkes for the precious assistance in editing this manuscript.

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