Summary
Post-transplant diabetes mellitus (PTDM) has emerged as a major adverse effect of immunosuppressive drugs (ISD). As recipients of organ transplants survive longer, the complications of diabetes mellitus have assumed greater importance. The predominant factor for causing PTDM by corticosteroids seems to be the aggravation of insulin resistance, however several studies have displayed deleterious effects on insulin secretion and $\beta$-cells. Calcineurin inhibitors induce PTDM by a number of mechanisms, including decreased insulin secretion and a direct toxic effect on the pancreatic $\beta$-cells. Recent in vitro studies stress on the increased apoptosis of $\beta$-cells when exposed to these drugs. Studies involving other immunosuppressive agents (mycophenolate mofetil [MMF], sirolimus) are scarcer and lead to conflicting results, while daclizumab seems to have a neutral effect. Clinical studies have consistently shown a greater potential of tacrolimus to induce PTDM compared with cyclosporine. Reducing PTDM incidence is a feasible goal while using corticosteroid-sparing regimens and/or lower tacrolimus trough levels. In patients developing PTDM, conversion from tacrolimus to cyclosporine could improve or reverse glucose tolerance abnormalities. In the absence of well-designed studies in this specific indication, treatment of PTDM is based on the same principles as type 2 diabetes mellitus. Thiazolidinediones do not display any pharmacological interaction with calcineurin inhibitors, but their safety and efficacy in PTDM need to be confirmed in large-scale randomized trials. Use of sulfonylureas has to be cautious regarding the suspected interaction of some of them with calcineurin inhibitors. If needed, immunosuppressive regimens have to be adapted to each patient on the basis of predictive criteria for the development of PTDM.

Key-words: Post-transplant diabetes mellitus • Immunosuppressive drugs • Tacrolimus • Cyclosporin • Corticosteroids • Review.
New-onset diabetes mellitus after solid organ transplantation is frequent [1-3] and associated with increased morbidity and mortality [4]. Among several risk factors predisposing to post-transplantation diabetes mellitus (PTDM) [4], the type of immunosuppression plays a major role, accounting for 74% of the variability of 12-month cumulative incidence of PTDM cases in a systematic review of the literature up to 2000 [5], with inclusion of corticosteroids and/or high-dose cyclosporine (CsA) or tacrolimus being the main risk factors. Even if insulin resistance is a major contributor to the physiopathology of PTDM, we will focus on the effects of immunosuppressive drugs (ISD) on the pancreatic β-cell function. The challenge for the transplant teams is to reduce the diabetogenicity of the immunosuppressive regimens and decrease the cardiovascular risk of the recipients, while containing a low rate of acute and chronic rejections.

ISD implicated in diabetes mellitus

Corticosteroids

In vitro and animal studies

If the role of corticosteroids in increasing hepatic glucose production, through gluconeogenesis stimulation, and peripheral insulin resistance has been established for a long time [6], and is considered to be the main contributor of their diabetogenicity, more recent studies have underlined their deleterious effects on insulin secretion [7, 8]. Several mechanisms, displayed in vitro studies on murine β-cells or human cell lines incubated with dexamethasone, have been proposed: insulin secretion inhibition by increased expression of α1-adrenergic receptors [9, 10], decreased cAMP levels [11, 12], GLUT2 protein decrease at the β-cell plasma membrane [13, 14], downregulation of glucokinase mARN [15], increased voltage-gated K+ channel activity [16], β-cell apoptosis through the activation of the calcineurin phosphatase and the corticosteroid receptor [17]. In this last study, dexamethasone-induced β-cell apoptosis was inhibited by the GLP-1 analogue, exendin-4 [17]. Whether a treatment with GLP-1 analogue could prevent or decrease the severity or reverse corticosteroids-induced diabetes would deserve to be studied.

Calcineurin inhibitors

The diabetogenicity of calcineurin inhibitors has been demonstrated, in both animals and humans, to be mediated through suppression of pancreatic insulin secretion [7, 18-21].

In vitro and animal studies

Morphologic abnormalities, including nuclear inclusions, cisternal dilatation of both the rough endoplasmic reticulum and the Golgi apparatus, vacuolisation [22], severe cytoplasmic degranulation and degeneration of islet β-cells [23], and cell death are observed with both calcineurins inhibitors in rodent β-cells.

Furthermore, both calcineurin inhibitors impair insulin gene transcription regulation [24] through inhibition of calcineurin signalisation [25-29]. Other mechanisms have been proposed: closing of the ATP-sensitive potassium channel [30], interference with mitochondrial function of pancreatic β-cells (CsA) [31], impairment of glucose-stimulated insulin secretion downstream of the rise in intracellular Ca2+ at insulin exocytosis [32], reduced ATP production and glycolysis derived from reduced glucokinase activity [33], decreased islet cell viability by a downregulation of anti-apoptotic factors and an accumulation of pro-apoptotic mediators [34] in cultures of freshly isolated human islets. A very recent work by Heit et al. demonstrated the crucial role of the calcineurin phosphatase regulatory subunits, calcinulin b1, in regulating multiple factors that control growth and hallmark β-cell functions in mice [35]. β-cell-specific deletion of this subunit led to age-dependent diabetes characterized by decreased β-cell proliferation and mass, reduced pancreatic insulin content and hypoinsulinaemia. Moreover, β-cells lacking Cnb1 have a reduced expression of established regulators of β-cell proliferation.

Very interestingly, a report of D’Amico E et al. demonstrates that GLP-1 is capable of preserving β-cell function and protecting cells from apoptotic cell death in mouse isla- noma cells exposed to a cocktail of ISD [36].

In summary, if both calcineurin inhibitors alter insulin secretion by several mechanisms, the effects of tacrolimus seem to be more profound and intense compared with the CsA-induced ones. One possible explanation could come from that the tacrolimus specific binding protein, i.e. FKBP-12, is preferentially located in β-cells, leading to a strong concentration of the drug in these cells. In contrast, the CsA specific binding site (ciclophiline) is preferentially located in the heart, the liver and the kidneys.

Clinical studies

In spite of some conflicting results [37], clinical studies have confirmed the deleterious effects of tacrolimus on insulin secretion. Boots et al. have examined the respective effects of steroids and tacrolimus in 15 non diabetic kidney transplant recipients, using IVGTT [38]. After withdrawal of 10 mg of prednisolone, insulin resistance significantly decreased. After tacrolimus trough level reduction from 9.5 to 6.4 ng/ml, pancreatic β-cell secretion capacity significantly improved, along with a HbA1c improvement, from 5.9 to 5.3% (p=0.002). Strumph et al. studied seven non-diabetic, non-transplanted subjects who were to receive FK506 for autoimmune diseases. All subjects underwent two standard oral glucose tolerance tests and two 180-min hyperglycaemic clamps immediately before and 10 weeks after starting FK506. FK506 decreased insulin secretion, regardless of initial glucose tolerance, while insulin sensitivity did not change.
It has also been reported a correlation between tacrolimus blood levels and PTDM incidence in kidney transplant recipients [39] as well as a statistically significant negative correlation between CsA concentration and insulin, proinsulin, C-peptide blood levels and a statistically significant positive correlation between CsA and glucose blood level in heart transplant recipients who developed hyperglycaemia after transplantation [40].

Striking features observed in most of the above-mentioned studies when they have been examined, are the dose-dependence and the reversibility of the deleterious effects on calcineurin inhibitors on the β-cells [41]. These findings are, of course, of paramount importance from a clinical point of view.

Other ISD: mycophenolate mofetil (MMF), sirolimus, daclizumab

The effects of ISD other than calcineurin inhibitors or corticosteroids are fairly less documented. Furthermore, the results of the studies are quite discordant.

In vitro and animal studies

Insulin secretory parameters and insulin gene expression of cultured human islets have been studied in the presence of ISD [19]. In opposite to FK506 and CsA, MMF had no deleterious effects. Other experimental in vitro and in vivo studies conclude to the neutral or beneficial role of sirolimus [42]. By contrast, Paty et al. exposing HIT-T15 cells and Wistar rat islets to various concentrations of five immunosuppressive agents found that glucose-stimulated insulin secretion was significantly inhibited after exposure to MMF and sirolimus, even more that after exposure to CsA or tacrolimus [7]. No reduction in insulin secretion was observed after exposure to daclizumab. In another study [34], MMF and sirolimus were able to decrease islet cell viability by downregulate anti-apoptotic factors in cultures of freshly isolated human islets. Other studies have displayed deleterious effects of sirolimus on MIN-6 cells and rat islets, but at supra-therapeutic concentrations [43].

Clinical studies

In a recent work [44], Italian authors have investigated the effect of the withdrawal of calcineurin inhibitors and the switch to sirolimus on peripheral insulin resistance and pancreatic β-cell response in 41 kidney transplanted patients: 26 in whom CsA was converted to sirolimus and 15 who were treated with sirolimus and tacrolimus for the first three months after grafting and then with sirolimus alone. Based on the results of OGTT and IVGTT before and six months after conversion to sirolimus-alone therapy, the withdrawal of anti-calcineurins was associated with a significant fall of insulin sensitivity (P=0.01) and with a significant defect in the compensatory β-cell response, as measured by the disposition index. These deleterious effects significantly correlated with the change of serum triglyceride concentration after the conversion to sirolimus-based therapy. Clinically, the switch to sirolimus was associated with a 30% increase of impaired glucose tolerance incidence and with the occurrence of four de novo diabetes.

By contrast, in the randomized PROGRAF Study, there was no difference of PTDM incidence in steroid and tacrolimus-treated kidney recipients whether they received sirolimus or MMF (about 7% at six months) [45].

Altogether, and from a pancreatic β-cell point of view, these data would point to daclizumab for the induction of immunosuppression and for the use of MMF instead of sirolimus as the ideal immunosuppressive regimen. Whether the use of MMF could allow to decrease the diabetogenic tacrolimus trough level as the same extent as sirolimus remains to be established. Furthermore, no clinical data has validated this theoretical assessment yet.

Clinical trials comparing the diabetogenic effects of CsA and tacrolimus after transplantation

Comparison of the respective effects of the two calcineurin inhibitors in inducing PTDM has been hampered for a long time in the absence of prospective randomised clinical trials. Several recently published works have however confirmed the former suspicion raised by retrospective analysis [46] on the higher incidence of PTDM in tacrolimus-treated patients. In the 6-month, open-label, randomized, prospective multicenter DIRECT study, tacrolimus and CsA were compared in 567 non-diabetic kidney graft recipients [47]. PTDM or new impaired fasting glucose occurred in 26.0% of CsA-treated patients and 33.6% of tacrolimus-treated patients (P=0.046). This increased risk of PTDM of solid organs (kidney, liver, heart, lungs) with tacrolimus has also been documented in a meta-analysis of 56 prospective and randomised clinical trials [48] The PTDM incidence was 16.6% with tacrolimus vs 9.8% with CsA, without any difference according to the transplanted organ.

However, in protocol-driven studies, steroid doses are comparable in both treatment arms, while in clinical practice, steroid dose used in conjunction with tacrolimus or CsA may differ. A retrospective study analysed renal transplant recipients without pre-existing diabetes receiving tacrolimus (n=100) or CsA (n=100) for whom one-year follow-up data were available [49]. Although tacrolimus-treated patients received a significantly lower cumulative dose of corticosteroids over the first three months post-transplant, significantly more tacrolimus-treated patients had new-onset diabetes than CsA-treated patients at 3, 6, 9 and 12 months. At 12 months, 18 patients receiving tacrolimus and two receiving CsA had diabetes (P<0.0001). After stratifying patients by age group, the frequency of diabetes was significantly higher with tacrolimus than with cyclosporine.

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in any age group. These results confirm that new-onset diabetes is strongly and significantly associated with tacrolimus vs CsA in renal transplant recipients, even when steroid dosing is lower with tacrolimus. This higher risk of PTDM with tacrolimus is amplified in HCV+ recipients (2.5 to 7 fold higher risk) and may be restricted to these patients [50]. Although the mechanism underlying the strong association between tacrolimus-induced PTDM and HCV+ remains quite elusive, the hypothesis stands on the increased insulin resistance in HCV patients [51-54]. The maximally compensated pre-existing insulin levels, allowing to maintain euglycaemia in face of HCV-induced insulin resistance, are suppressed after immunosuppression with tacrolimus, resulting in the development of PTDM.

However, in the randomized LIS2T study comparing CsA (n=250) and tacrolimus (n=245) in liver transplant recipients according to the VHC status, 14% of tacrolimus-treated patients vs 6% of CsA-treated ones developed PTDM at month-9 post-transplant [55].

**Effect of the dose of tacrolimus**

There was a progressive decline in the incidence of PTDM induced by tacrolimus-based regimens, from 20% in the early 1990s to 0-5% most recently [56]. The low incidences of PTDM were achieved with those protocols employing lower blood levels of tacrolimus and/or corticosteroid elimination. In these studies, the risk of developing PTDM was not increased in comparison with CsA-based therapy. These results emphasize the importance of reducing the immunosuppressive medication load and the role of corticosteroids in the development of PTDM.

**ISD and islet transplantation**

The deleterious effects of ISD on β-cells have also to be taken in consideration in islets transplantation. Assuming that neogenesis contributes to the long-term function of islet grafts, Gao et al. have studied the effects of ISD on precursor cell proliferation and differentiation [57]. Examining the effects of clinically used doses of ISD on freshly isolated human pancreatic cells, they showed that MMF has a potent inhibitory effect on human islet neogenesis primarily through an antiproliferative effect on the precursors, whereas tacrolimus mainly affects β-cell differentiation. Sirolimus and daclizumab have no adverse effects on these parameters. These data are consistent with the dramatic improvement of islet grafts with the immunosuppressive Edmonton protocol [58].

However, in spite of reaching long-term insulin-independence and HbA1c normalisation, successful islet cell transplantations are characterized by an altered insulin secretion profile with a decrease or absent first phase insulin response after a glucose load [59]. Although the causes of these insulin secretion abnormalities could be multifactorial, with suboptimal islet number, low engraftment, chronic rejection, loss of islet-acinar integrity, heterotopic site, denervation, or insulin resistance, avoidance of diabetogenic immunosuppression is pivotal to enhance outcomes of clinical islet transplantation [60].

**Reduction of PTDM**

**Effect of corticosteroid-sparing regimen on PTDM**

In the pre-CsA era, chronic high-dose steroid therapy was a major contributing factor to the development of PTDM [61-63]. However, even if steroid contribution to PTDM has since decreased, a still valid statement is that the rate of occurrence of PTDM falls significantly when using corticosteroid sparing protocols. Compared with CsA-based regimen, immunosuppression using tacrolimus appears to decrease the rate of acute rejection episodes [64, 65], allowing to withdraw steroids 3 to 6 months after renal transplantation [66-68]. Boots et al. have investigated the hypothesis of the safety of even earlier steroid withdrawal after transplantation [69]. Sixty-two patients treated with tacrolimus were prospectively randomized to stop 10 mg of prednisolone after day 7 posttransplantation (STOP) or to gradually taper steroids in three to six months (TAP). While there was no difference between the two groups after a median follow-up of 2.7 years concerning patient and graft survival, incidence and severity of acute rejections and of renal function, the incidence of PTDM (defined as the use of antidiabetic medication) was 8.0% in the STOP group and 30.3% in the TAP group (P=0.04). Furthermore, all cases occurred after one year in the STOP group, raising the question of the relationship with immunosuppressive regimen, while all cases of the TAP group occurred in the first four months (P<0.001). Recently, Rostaing et al. showed that by employing induction therapy with daclizumab plus concomitant therapy (MMF + tacrolimus) in 538 kidney transplant recipients, it is possible to taper tacrolimus blood concentrations rapidly and completely avoid the use of maintenance corticosteroids [70]. At 6 months post-transplant, this regimen resulted in a significant reduction in the incidence of PTDM compared with the standard arm, which employed tacrolimus, MMF and concomitant corticosteroid (P<0.001) (table I). A quite similar study in living transplant recipients compared the effects of daclizumab induction + tacrolimus, without corticosteroids, with progressively tapered prednisone + tacrolimus [71]. At 3 months post-transplantation, the incidence of PTDM (defined by insulin therapy > 30 days) was 3-fold higher in the steroid group (17.8% vs 5.1%; P<0.001). A single-center study conducted in Minneapolis, involved 349 kidney transplant recipients [72]. The induction immunosuppression, including thymoglobulins and steroids, was stopped at day 5. For the maintenance immunosuppressive regimen, patients were randomised for the calcineurin inhibitor (tacrolimus vs
CsA) and between sirolimus and MMF. At 3 years post-transplantation, without any steroid treatment in 84% of the patients, only four (1.8%) had developed a PTDM, all in the tacrolimus-sirolimus arm and within the first 6 months. The results of a subanalysis of all 12 European kidney studies [56] whereby the tacrolimus-based, corticosteroid-free treatment arms were compared with the reference tacrolimus-based, corticosteroid-containing treatment groups were consistent with those reported by Rostaing et al. [70].

Altogether, and since the use of low doses of tacrolimus, these data underline the major role of corticosteroid in inducing PTDM.

Recent clinical studies in liver transplantation have reported safety advantages and similar acute rejection rates with early steroid withdrawal. The aim of this French study was to evaluate the efficacy and safety of an immunosuppressive regimen with steroid withdrawal at day 14 in a multicenter, 1-year, comparative, double blind, placebo-controlled design. All patients received basiliximab + CsA + intravenous methylprednisolone, and they were randomized at day 7 to receive a maintenance regimen with CsA + prednisolone (group 1; n=90) or without steroids (CsA + placebo; group 2; n=84). While fewer patients received an antidiabetic treatment in the placebo group (2 vs 10), the incidence of acute rejection at 6 months was 38.1% in group 2 vs 24.4% in group 1 (P=0.03) [73]. If this study confirms the beneficial role of early steroid withdrawal on the glucose tolerance in liver transplant recipients, CsA-based maintenance immunosuppression is obviously not safe enough and would need to be reinforced by non- or less diabetogenic ISD, such as MMF, to avoid a higher incidence of acute rejection.

Effect of conversion from tacrolimus to CsA

Even if several PTDM risk factors have been determined, the individual risk assessment remains elusive and most of the transplantation teams do not base their immunosuppression choice on that single risk in pre-transplantation non-diabetic patients. Instead, some authors have studied the conversion of tacrolimus to the other calcineurin inhibitor, CsA. Bouchta et al. retrospectively analysed the outcome of the glucose metabolism after conversion to CsA in 34 renal transplant patients who developed PTDM under tacrolimus treatment [74]. HbA1c levels decreased from 6.8 ± 0.8% at conversion to 6.0 ± 0.6% at 12 months. From 11 patients receiving insulin before conversion, three could stop it, and the insulin dose was reduced in seven. The average daily insulin dose among these patients was reduced from 31 ± 17 units at conversion to 13 ± 12 units at 12 months (P<0.05). Diabetes reversed (fasting plasma glucose ≤ 126 mg/dL without therapy) in 44% of patients during the first year after conversion (P<0.001).

This study, like others [75-78], does not provide the definitive proof of a causal association between conversion and improved glucose metabolism. Indeed, spontaneous reversals of PTDM in tacrolimus-treated patients have been previously reported [79]. These “spontaneous” reversals can, in particular, be explained by the ongoing tapering of steroid doses along with the conversion. However, several arguments suggest that conversion contributes to the observed benefits. The maximum reduction in glycaemia and insulin requirements occurs rapidly after conversion. In addition, in patients who already have prednisolone reduced to maintenance doses before conversion, the improvement of glycaemia and HbA1c levels is of similar timing and magnitude as in patients who have a slight reduction of their prednisolone dose after the conversion [74, 75].

Although this review focuses on glucose metabolic effects of immunosuppressive agents, it is important to notice that calcineurin inhibitors have distinct consequences on other cardiovascular risk factors. While tacrolimus has hardly any effects on lipid levels and blood pressure, CsA is known to cause hyperlipidaemia and arterial hypertension [80]. However, these risk factors can usually be efficiently controlled more easily than diabetes [74].

**Treatment of ISD-induced diabetes**

As for the physiopathology and risk factors which are very similar to those of type 2 diabetes, objectives and modalities of the treatment of PTDM are not different from the usual management of type 2 diabetes. We will not detail the

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**Table I**

Incidence of acute rejection and PTDM with tacrolimus-based regimens [68].

<table>
<thead>
<tr>
<th></th>
<th>Tacrolimus + MMF + corticosteroid (n=278)</th>
<th>Tacrolimus + MMF + daclizumab (n=260)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy-proven acute rejection (%)</td>
<td>16.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Corticosteroid-resistant acute rejection (%)</td>
<td>4.3</td>
<td>5.0</td>
</tr>
<tr>
<td>PTDM (%)</td>
<td>5.4</td>
<td>0.4*</td>
</tr>
</tbody>
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MMF = mycophenolate mofetil; PTDM = post-transplantation diabetes mellitus. * p=0.001 for the difference between the two treatment groups.
guidelines in this review [81], in the absence of specific randomized studies in this population. We will focus on some studies that have evaluated the safety and drug interaction of some oral anti-diabetic agents in transplanted patients.

The safety of thiazolidinediones have been studied in PTDM in 10 patients treated with pioglitazone for a mean of 242 days [82] and in 18 patients receiving rosiglitazone for a mean duration of 381 days [83]. The addition of pioglitazone caused no significant changes in mean tacrolimus [82, 83] or CsA doses [83]. These absence of drug interaction has been confirmed in 22 renal transplant patients with PTDM who received rosiglitazone therapy [84]. In this study, fifteen patients were treated with tacrolimus and seven patients with CsA. There were no changes in CsA and tacrolimus blood levels. In this study, one patient had to stop rosiglitazone because of edema after 5 days [83]. In another study, 40 consecutive patients with PTDM after liver or kidney transplantation received 4 mg of rosiglitazone, in addition to insulin in 33 of them [85]. After a mean follow-up of 26 weeks; insulin was able to be discontinued in 30/33 (91%) patients; 25/40 (63%) continued on 4 mg/d of rosiglitazone, and 15/40 (37%) required an increase to 8 mg/d. Mild edema developed in 13% of patients; significant weight gain did not occur.

These preliminary results suggest that thiazolidinediones are safe oral agents for the management of PTDM, but further large-scale evaluations are required for both evaluate the efficacy in randomised studies and confirm the safety concerning the risk of heart failure [86] in these at risk population [87].

With regard to the sulfonylureas, co-administration of CsA and glibenclamide in six post-transplant diabetic patients resulted in a 57% increase in the steady-state plasma CsA levels, despite normal hepatic and renal functions in the patients [88]. This elevation in CsA level is possibly due to an interaction between the two drugs resulting from an inhibition of CYP3A4-mediated metabolism of CsA by glibenclamide. In contrast, glipizide does not interfere with CsA pharmacokinetics in renal allograft recipients [89].

At last, insulin regimens in transplant patients with a corticosteroid-induced glycaemic profile (hyperglycaemia during the day and in the evening with fasting normo- or hypoglycaemia) has to be adapted in order to avoid nocturnal hypoglycaemic episodes [90].

Conclusions and prospects

PTDM has emerged as a major adverse effect of ISD. As recipients of organ transplants survive longer, the long-term complications of diabetes mellitus have assumed greater importance.

While the cellular and molecular mechanisms involved in ISD-induced diabetes are better explained, the reduction of PTDM and of its well-documented impact on survival and functional outcomes warrant efforts to develop immunosuppressive regimens and drugs that eliminate or reduce the need for corticosteroids and calcineurin inhibitors without jeopardising graft function. Until methods for inducing specific graft tolerance become available, immunosuppressive regimens should be tailored to the individual patient on the basis of predictive criteria for the development of PTDM. Finally, the tools for reaching a tight glycaemic control exist and should be use more aggressively. In the near future, the specific properties of incretin-based therapies, including the decrease of β-cell apoptosis and the stimulation of their proliferative capacities, have to be assessed in the treatment of PTDM. In addition, comprehensive care of transplant recipients must include attempts to reduce other cardiovascular risk factors such as hypertension, smoking, dyslipidaemia, and obesity through a multidisciplinary team approach.

References


