Immunology of pancreatic islet transplantation

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SUMMARY
Clinical protocols in type 1 diabetic patients to optimize islet survival and function post-transplantation improved dramatically in the last decade, but it is clear that this approach still has potential limitations to provide long term insulin independency. Islet allografts administered in the liver via the portal vein are exposed to several factors contributing to a rapid loss of function that may reach 50% of the initial beta cell mass. Allo- and auto-immune reactions – an unique situation in clinical transplantation - are partially overcome with immunosuppressive regimen. Serological markers and T cell reactivities may correlate with graft failure. Most of the drugs that are used, including rapamycin (sirolimus) or the calcineurin inhibitor tacrolimus (FK506), have deleterious effects on beta function and/or insulin sensitivity. Immediate factors that limit initial islet engraftment have been elucidated, including instant blood mediated inflammatory reaction and angiogenesis. Newer interventions designed to promote islet growth and to protect islets in the long run from immunological injury are rapidly approaching clinical trials.

Key-words: Islets of Langerhans • Transplantation • Immunosuppression • Immunosuppressive treatment • Sirolimus • Calcineurine • Tacrolimus • Review.

RéSUMÉ
Immunologie de la greffe d’îlots de Langerhans
Les récents programmes de greffes d’îlots chez les diabétiques de type 1 ont démontré des progrès considérables, mais il reste des limites pour assurer une fonction sur le long terme et une insulino-indépendance. Les allogreffes d’îlots injectés par voie portale dans le foie sont exposés à de nombreux facteurs d’agressions, expliquant une perte de fonction estimée à 50 % dans les premiers jours de la greffe. Les réactions allogéniques et autoimmunes, une situation unique en transplantation, sont partiellement contrôlées par l’immunosuppression chronique. La plupart des immunosupresseurs utilisés, comme la rapamycine (sirolimus) ou les inhibiteurs de la calcineurine tel le tacrolimus (FK506), ont des effets délétères sur la fonction des cellules bêta et/ou sur la sensibilité à l’insuline. Des facteurs immédiats peuvent limiter la viabilité des îlots, comme une réaction immédiate de thrombose et la réduction de l’angiogenèse. De nouveaux traitements qui ont comme objectifs la survie des îlots et/ou la réduction de l’apoptose sont attendus.

Mots-clés : Greffes d’îlots de Langerhans • Immunosuppression • Traitement immunosupresseur • Sirolimus • Calcineurine • Tacrolimus • Revue générale.
Introduction

Optimised insulin injections remain the mainstay life-sustaining therapy for patients with type 1 diabetes (T1D) in 2006. However, a small subset of patients with T1D are very sensitive to insulin and lack counter-regulatory measures, putting them at higher risk of neuroglycopenia. Patients have been enrolled in islet cell transplantation programs with significant improvements during the last few years. In a landmark study published in 2000, Shapiro et al. [1] reported seven consecutive patients treated with islet transplants, all of whom maintained insulin independence for one year. The approach included: (i) selection of T1D subjects for islet alone who suffered from severe hyperglycaemia unawareness or labile diabetes; (ii) the immunosuppressive protocol was steroid-free, consisting of dacluzimab (an anti-CD25 monoclonal antibody) induction, the anti-rejection drugs sirolimus and calcineurin inhibitor tacrolimus; (iii) islets were prepared for transplant in the absence of xenogeneic proteins using human albumin rather than bovine albumin; and (iv) 10,000 IEs (islet equivalents)/kg of body weight was the minimum islet transplant administered to each patient, often administered as two or sometimes three infusions, from sequential donors. Since the original report of the Edmonton protocol in 2000, an estimated 500 islet transplants have been conducted worldwide using variants and further advances [2], including 50 in France. Clinical results of these grafts were encouraging although less efficient than the original Edmonton study with 30 to 60% insulin-independent patients at one year. These half tone results reinforced the need for better understanding of the underlying cellular mechanisms associated with the reintroduction of functional beta cells in long-standing T1D patients and on the possible immune factors implicated in the loss of function or destruction of islet grafts. Approximately 80% of grafts continue to function and secrete C-peptide however, and patients benefit considerably from near-normal HbA1c and avoidance of hypoglycaemic reactions. The exact cause for progressive islet dysfunction is incompletely defined to date, but most likely reflects multifactorial aetiologies, including not only acute and chronic rejection, but the recurrence of autoimmunity and the fact that islets are placed in a non-physiological environment and are exposed further to chronic drugs that have diabetogenic and antiproliferative side effects. Furthermore, islet exhaustion may set in sequentially, forcing the respective contribution of the immunosuppression regimen, immediate pro-apoptotic factors during islet engraftment, allogeneic immune response and recurrence of autoimmunity in beta cell loss.

Immunosuppression

Early strategies relied on protocols that had proven success in solid organ transplantation which consisted of azathioprine, cyclosporine and corticosteroids. Under these protocols, fewer than 10% of patients were able to achieve insulin-independence [2]. The Edmonton protocol (and more recent variants) use a glucocorticoid-free combination of immunosuppressive agents, typically including Dacluzimab (anti-CD25 mAb) administered in the peri-transplant period, sirolimus and tacrolimus which are administered to prevent chronic rejection. Regular monitoring of sirolimus and tacrolimus levels ensures adequate and effective immunosuppression and avoids unnecessary overdosing, which could result in rapid and severe toxicities. Although the current immunosuppressive therapies have improved outcome in transplant recipients at 1- and 3-year time points post-transplantation, it is now emerging that insulin-independence is not sustainable in most subjects once they reach 5 years post-transplantation [3]. Rapamycin (sirolimus) is a macrolide fungicide with immunosuppressant properties, which may cause post-transplant diabetes mellitus. Rapamycin had a dose-dependent, time-dependent, and glucose-independent deleterious effect on MIN-6 cell viability [4]. A supra-therapeutic rapamycin concentration of 100 nmol/l had a deleterious effect on the viability of rat and human islets, causing apoptosis of both alpha- and beta-cells. Last, studies in Sprague-Dawley rats have demonstrated that rapamycin, with its known antiproliferative properties, is also associated with insulin resistance [5] and when combined with FK506 induces diabetes. Tacrolimus (FK506) time-dependently suppressed glucose-stimulated insulin secretion from rat islets, and at a therapeutic concentration of 0.01 micromolar/liter, it suppressed glucose-stimulated insulin secretion to 32 ± 5% of the control value after 7-day incubation [6]. Most of these in vitro effects were reversible after drug withdrawal. Therefore, chronic immunosuppression with calcineurin inhibitors has proven its efficacy to limit T cell activation, but may seriously affect long term function of islet transplants [7, 8]. The need for calcineurin inhibitor-free immunosuppressive regimen appears a high clinical priority as well as strategies for long-term tolerance induction. New immunosuppression protocols are planned. T cell depleting agents such as alentuzumab (Campath-H, anti-CD52mAb) which as shown its efficacy in the management of autoimmune diseases [9], as well as several compounds that bind to CD80 and CD86, blocking the interactions with the T cell co-stimulatory receptor CD28, such as the analogue LEA29Y (Belatacept) [10] are promising. An alternative approach to traditional immunosuppression which has targeted lymphocyte activation is to inhibit lymphocyte migration to their site of activation. Emerging compounds of interest include FTY720 a potent inhibitor of lymphocyte egress from the thymus and lymph nodes which as shown promising results in islet transplantation in non human primates [11] and in autoimmune diabetes prevention in NOD mice.

Immediate islet engraftment

An important and rapid tissue loss is associated with islet transplantation, which explains the need to graft large numbers
of islets from different donors. Human islets exposed to human blood trigger an “instant blood mediated inflammatory reaction”, IBMIR, characterized by platelet consumption and activation of the coagulation and complement systems [12]. Interestingly, human islets express tissue factor (TF), an integral component in the coagulation cascade [13]. Its role in this adverse clotting reaction is suspected. The islets become surrounded by clots and infiltrated with leukocytes with evidence of islet damage. Addition of heparin reduces IBMIR and islet damage. TF and MCP-1 (macrophage chemoattractant protein) expression in human islets can also be decreased by adding nicotinamide to the culture medium [14]. These encouraging results explained why nicotinamide is used in a non randomized fashion in most islet transplantation programs. Intraperitoneally transplanted islets are avascular at the time of transplantation and take up to 14 days to fully revascularize [15], during which time, up to 60% of islet mass may be lost. This complex glomerular-like network of blood vessels which coalesce at the periphery or traverse the central core of the islets is destroyed after enzymatic digestion. The antiproliferative effects of sirolimus may theoretically be disadvantageous both for angiogenesis in newly transplanted islets [16, 17] and for islet neogenesis from ductal stem cells [18]. Ischemia–reperfusion and coagulation-thrombosis lead to inflammation, and islets are very susceptible to injurious effects of activated macrophages and proinflammatory cytokines.

**Recurrence of autoimmunity**

Type 1 diabetes results from a selective destruction of the beta cells by an autoimmune mechanism. Results from twin to twin pancreas transplantation have underlined the importance of the recurrence of autoimmunity with the presence of memory T cells with CD8+ T cells that rapidly infiltrate the islets and destroy the beta cells [19]. We have previously reported in 68 C-peptide-negative diabetic patients receiving pancreatic allografts, that chronic graft failure was associated with positivity of both antibodies to GAD65 and IA-2 [20]. When immunosuppression was not adapted, rapid destruction of islet grafts were observed with a sudden rise in GAD65 and IA-2 autoantibody titers [21]. Recent pathologic observations in a patient with long-standing diabetes have shown the persistence of T cells and macrophages at the vicinity of insulin positive cells in the exocrine tissue, which limit beta cell regeneration [22]. These observations, clearly illustrate, the necessity of an efficient immunosuppression to block recurrence of autoimmunity. The predictive value of islet cell antibodies is a matter of controversy, since immunosuppressive regimen are unable to control perfectly anti-pancreas antibody production without immediate metabolic consequence. Direct analysis of islet cell infiltrates is needed to assess autoimmune recurrence through access to islet tissue or specific imaging procedures such as injection of magnetic labelled T cells and MRI [23].

**Allograft rejection**

The role of pretransplant sensitization to human leukocyte antigen (HLA) in islet transplantation is crucial, in theory due to the multiple sources of tissue donors. A recent study addressed this question and concluded that humoral and cellular sensitization to histocompatibility antigens, prior to and after islet transplantation, are associated with the failure of transplanted islets [24]. Rapid failure (<3 weeks) in three cases was accompanied by increases in precursor frequencies of graft-specific alloreactive T-cells [25]. T-cell reactivities in peripheral blood can therefore be used to monitor immune mechanisms, which influence survival of beta-cell allografts in diabetic patients. Improvements in purity, yield and viability of islet preparations are rendering single donor islet transplants sufficient for insulin independence [26]. Living donor islet transplantation is another strategy to use more strict criteria of HLA matching without the haemodynamic instability and pro-inflammatory cytokines that are common in non-heart-beating and brain-dead donors and to reduce warm and cold ischaemia time [27].

**Protecting islets from immunological injury through beta cell growth**

Expanding stem/progenitor cells and then to convert them into beta cells by treatment with GLP-1 [28], reducing beta cell apoptosis, are additional strategies to prevent or limit the initial beta cell loss. Access to GLP-1 receptor agonists (exenatide) in clinics, have led to promising results in open trials which necessitate confirmation in randomised trials. Recent observations in NOD mice with gastrin + EGF therapies [29] are very encouraging, with the increase in islet cell mass and prevention of autoimmune diabetes. These approaches, if confirmed in humans, may play a central role in the future of islet transplantation.

**Conclusion**

Re-exposure of type 1 diabetic patients to living allogeneic beta cells is a complex but fascinating model of experimental immunology. Controlling both allo- and autoimmune responses is challenging. Novel immunosuppressive and inflammatory blockade agents in the field of islet transplantation have made significant improvements. Those agents should be non-diabetogenic or reduce the need for more diabetogenic immunosuppressive agents, reduce initial damage of islet cells and promote engraftment, induce a functional tolerance, and aim to manage autoimmunity, in addition to stopping allograft rejection processes. In the future, special emphasis will be placed on new immunotherapeutic strategies, as a means to produce tolerance to islet allografts without the spectrum of islet toxicity, and on additional pharmaceutical interventions to promote islet cell growth.
References


