

Review

Immunosuppression for Islet Transplantation

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The development by the Edmonton group of a sirolimus-based, steroid-free, low-tacrolimus regimen is a significant breakthrough that allows the rate of insulin independence after islet transplantation to increase from 13% to 80% at 1 year; however, the rate is reduced to 50% at 3 years, attributed to prolonged tacrolimus exposure. Recently, immunosuppression agents such as cyclosporine, mycophenolate mofetil, and the novel agent FTY 720 have been used instead of tacrolimus. Lymphocyte-depleting antibodies such as anti-thymocyte globulin, alemtuzumab, and hOKT3γ1 (ala, ala) have been launched, and a costimulatory blockade of anti-CD40 monoclonal antibodies and CTLA4-Ig will be attempted in the near future. Moreover, the potential of a novel immunosuppressing peptide could now be realized using new technology called the protein transduction system. In this review, we show some of the most recent contributions to the advancement of knowledge in this field.

Key words: islet transplantation, steroid-free, Edmonton protocol, protein transduction system

In 1974, the first clinical allogeneic islet transplantation was performed at the University of Minnesota [1]. Immunosuppressive regimens depend heavily on steroids and azathioprine because of the previous appearance of calcineurin inhibitors such as cyclosporine. Unfortunately, no patient has achieved insulin independence with this procedure, and only a few cases have showed transient graft function, as evidenced by measurable serum C-peptide [1], and even when cyclosporine is available, there has not

been much improvement. In 1988, Camillo Ricordi developed an automated method for the isolation of human pancreatic islets [2]. This technique provided an opportunity for large numbers of human islets to be isolated and transplanted, leading to the first reports of insulin independence using cyclosporine-based regimens. [3, 4]

Immunosuppressive regimens in the 1990s evolved with the release of new compounds, but the rates of insulin independence reported by the International Islet Transplant Registry (ITR) culminated at a disappointing 13%. At the University of Geneva, an immunosuppression protocol consisted of cyclosporine, azathioprine, and steroids, and induction therapy was performed for a period of 14

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days with anti-thymocyte globulin until 1997. From 1998 to 2000, a new protocol consisted of cyclosporine, mycophenolate mofetil, and steroids, and induction therapy with basiliximab was performed on day 0 and 4. This is the first report of the use of an anti-interleukin-2-receptor monoclonal antibody in islet transplantation [5, 6]. The protocol was used in the initial experience of the Swiss-French GRAGIL consortium from 1999 to 2000. At the completion of a 12-month follow up, 0% primary nonfunction, 50% graft survival, and 20% insulin independence were observed [7]. The Giessen group reported remarkable results in the late 1990s, with approximately 30% of transplanted patients achieving insulin independence [8, 9]. The Milan group has reported that 35% of 20 consecutive patients receiving islets after kidney grafts achieved insulin independence [10]. Remarkably, the Giessen and Milan results were achieved with a conventional immunosuppressive regimen including steroids and cyclosporine, which has been preferred over tacrolimus because of its alleged lower islet toxicity.

In 2000, the Edmonton group reported extremely impressive advances in clinical islet transplantation. All 7 consecutive recipients of allogeneic islet grafts achieved insulin independence [11]. A subsequent update of their results reported an 80% actual rate of insulin independence at 1 year [12, 13]. This observation has led to renewed interest in islet of Langerhans transplantation as a means of curing diabetes, as clinical programs are being started at an increasing number of transplant centers throughout the world. The design of a sirolimus-based, steroid-free, low-tacrolimus regimen has fundamental to this progress.

Currently explored alternatives to tacrolimus include cyclosporine, mycophenolate mofetil, and the novel agent FTY 720 [14, 15]. To achieve tolerance, several strategies using lymphocyte-depleting antibodies (anti-thymocyte globulin, alemtuzumab, hOKT3 γ 1 (ala, ala)), or costimulatory blockade (anti-CD40 antibody, CTLA4-Ig) have been performed [16–19]. Moreover, the potential of a novel immunosuppressing peptide could now be realized, thanks to a new cell-delivery system [20]. Here, we review some of the most recent contributions to the advancement of knowledge in this field.

Immunosuppressant of the Edmonton protocol

The Edmonton protocol uniquely combines several strategies designed to specifically address the various obstacles encountered in the isolation, transplantation, and immunosuppression sequence. One of these strategies involves an improved immunosuppressive protocol consisting of sirolimus (rapamycin), low-dose tacrolimus, and anti-IL2-receptor monoclonal antibody (daclizumab) induction. The immunosuppressive protocol avoids the diabetogenic effects of glucocorticoids in islet transplantation. Although the mechanisms are not fully understood, the existence of glucocorticoid-induced hyperglycemia has long been acknowledged, and its detrimental effects on islet function *in vitro* and *in vivo* have been described [21, 22].

Calcineurin inhibitors such as tacrolimus have also been associated with impaired *in vitro* and *in vivo* islet graft function [22, 23]. Moreover, long-term studies have shown that short-term cyclosporine in dogs can result in the permanent loss of functionally competent islets [24]. A study of biopsies obtained from whole pancreas transplants in hosts treated with cyclosporine or tacrolimus resulted in observation of cytoplasmic swelling, vacuolization, and apoptosis as evidence of direct islet cell damage. The presence and extent of damage appears to correlate with high serum levels of calcineurin inhibitors and pulse steroid administration. The lesions are also more marked with tacrolimus than with cyclosporine therapy [25]. These observations provide a rationale for a glucocorticoid-free regime and for lowering the dosages of calcineurin inhibitors in the Edmonton immunosuppressive protocol.

Sirolimus has been shown to be a rather harmless agent in terms of islet toxicity. *In vitro* impairment of islet function is seen only at extremely high sirolimus concentrations. At doses 10 to 50 times the effective antirejection dosage, hyperglycemia has been observed in islet-transplanted animals without histological evidence of end-organ toxicity [26]. The synergism of sirolimus and calcineurin inhibitors allows their dosage to be reduced substantially, and thus islet toxicity, without increasing the occurrence of acute rejection episodes [27, 28]. Since both siroli-

mus and tacrolimus bind to FKBP-12, competition for FKBP-12 would prevent synergism [29]; however, *in vivo* observations in both animal models and humans suggest a strong potentiation of the efficacy of both drugs [30–32].

It appears that the actual rate of insulin independence is 80% at 1 year and 50% at 3 years in Edmonton patients. The reasons for the decrease in islet function after the first year are not well understood. Although the dose of tacrolimus is low, the long-term toxicity of tacrolimus to islets has been suspected. In addition to tacrolimus toxicity, relatively high doses of sirolimus (trough levels of 10–15 ng/mL for the first 3 months) have been associated with serious side effects. The most notable reported side effects of sirolimus are mouth ulcerations, dyslipidemia, and myelotoxicity. It is a matter of concern that such side effects are experienced by almost all patients.

Progressive immunosuppressive protocol and perspectives for the near future

After the Edmonton protocol report, several institutions have considered calcineurin inhibitor-free regimens to be an excellent way of avoiding drug-induced diabetogenicity and of minimizing the development of kidney toxicity in patients prone to developing diabetic nephropathy. The Minneapolis group has shown excellent results using tacrolimus as the initial immunosuppression, followed by the gradual replacement of tacrolimus with mycophenolate mofetil as maintenance immunosuppression beginning 1-month posttransplant [14]. The same group has also shown preliminary evidence of the safety and efficacy of corticosteroid and calcineurin inhibitor-free immunosuppression in a relevant preclinical transplant model [15]. Induction immunosuppression was carried out in this previous study with intravenous basiliximab, anti-IL-2 receptor blockade, which has been used in clinical islet transplantation from non-heart beating and living donors in our university instead of daclizumab [33, 34]. Maintenance immunosuppression was with everolimus, sirolimus analogue, and FTY 720. FTY 720 is a novel immunosuppressive agent that acts on lymphocyte homing and thus interferes with T cell-antigen cognate interaction. The availability of FTY 720 for phase II clinical trials of islet

transplantation is eagerly anticipated.

The use of lymphocyte-depleting agents during the induction period has been attempted with success in protocols based on tacrolimus-sirolimus association by the Minneapolis group. The use of anti-thymocyte globulin and the humanized anti-CD3 monoclonal antibody (mAb) hOKT3 γ 1 (ala-ala), lacking Fc-receptor-binding properties (and thus avoiding massive cytokine release by cross-linked macrophages) and with reduced immunogenicity, has resulted in high rates of insulin independence after single donor islet transplantation [16]. The Edmonton group is currently testing humanized mAb alemtuzumab (Campath-1H) in a group of islet transplant recipients. This compound targets the CD52 molecule, located on the cell surface of lymphocytes and monocytes, resulting in lasting and profound depletion of these lineages. Results equivalent to those obtained with the original Edmonton protocol have been reported [35].

Biological agents that block key T cell costimulatory signals have demonstrated extraordinary promise in animal models. Both CD28 and CD154 molecules are located at the surface of CD4(+) T cells and deliver costimulatory signals. Two types of costimulatory blocking agents, CTLA4-Ig preventing CD28-CD80/86 interaction and anti-CD154 preventing CD40-CD154 interaction, have reached the preclinical stage. In a nonhuman primate model, LEA29Y, a mutant CTLA4-Ig molecule with increased binding activity, sirolimus, and the anti-IL-2R regimen have significantly prolonged islet allograft survival [17]. Islet transplantation under the cover of anti-CD154 monotherapy consistently allows for allogeneic islet engraftment and long-term insulin independence in this highly relevant preclinical model [19]. From these studies, the concept of maintenance therapy with costimulatory blocking agents has emerged as a valid strategy for clinical islet transplantation. A clinical trial utilizing humanized anti-CD154 mAb in recipients of solitary islet transplants commenced in 1999; however, it was reported that unusual thromboembolic complications occurred in kidney transplant recipients receiving mAb in a concurrent trial [36, 37]. To circumvent this potential complication, an Emory University group developed a chimeric antibody targeting CD40 as an alternative to CD154. Anti-CD40 combined with LEA29Y dramatically facilitates long-term islet

allograft survival [18].

Development of immunosuppressive agents by protein transduction technology

An important mechanism whereby calcineurin promotes T cell activation and cytokine gene induction is largely attributed to a family of transcriptional regulators referred to as the nuclear factor of activated T cells (NFAT). Immunosuppressants cyclosporine A and FK506 inhibit the activity of calcineurin phosphatase on all its protein substrates, including NFAT [38, 39]. These drugs have revolutionized transplant therapy; however, the inhibition of calcineurin outside the immune system has a number of side effects including hyperglycemia, progressive loss of renal function, hypertension, neurotoxicity, and increased risk of malignancy [40-43]. In particular, the use of FK506 and cyclosporine A in human organ transplantation has been associated with a 10-30% incidence of diabetes [44].

In the search for safer drugs, we have developed a cell-permeable inhibitor of NFAT using the protein transduction system [20, 45-52]. The NFAT inhibitor peptide, VIVIT, has been developed based on the conserved calcineurin docking site of the NFAT family [53]. The peptide interferes selectively with calcineurin-NFAT interactions without affecting any of calcineurin's other targets. Therefore, VIVIT might be useful as a therapeutic agent that is less toxic than current drugs. The NFAT inhibitory peptide was covalently linked at its C terminus to a short stretch of arginine residues (11R-VIVIT). Polyarginine facilitates the uptake of peptides and protein into cells with high efficiency [20, 46-50]. This peptide has been observed to specifically and significantly inhibit NFAT function in a T cell line, and appears stable enough to survive in the circulation of a mouse model. Using a mouse model of diabetes, we investigated whether 11R-VIVIT can prevent transplant rejection. Following the transplantation of islet cells from fully mismatched mice, treatment with the peptide was observed to prolong graft survival, and the transplanted islet cells were still producing insulin 50 days later. Moreover, insulin secretion did not change at any concentration of 11R-VIVIT, whereas FK506 inhibited insulin secretion, and the amount

secreted decreased significantly. These results indicate that the NFAT inhibitor peptide is less toxic than calcineurin inhibitors with regard to insulin secretion [20].

Although the peptide is a long way from clinical trials, the above strategy provides interesting proof that the toxicity of calcineurin inhibitors can be reduced.

Conclusions

Immunosuppression is critical in islet transplantation because islet grafts are prone to immune destruction not only by allojection, but also by the recurrence of autoimmunity. New agents other than calcineurin inhibitors should be developed with the aim of solving diabetogenicity and nephrotoxicity problems. The next step should probably be the development of new agents other than sirolimus to avoid the problematic side effects of the drug. Safer immune suppressors will have benefits not only for transplant patients. If there are fewer side effects, the drugs could then be employed in treating autoimmune diseases such as psoriasis, for which current drugs are considered too dangerous.

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