Current Status of Clinical Islet Transplantation

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Islet transplantation is currently being explored as a treatment for patients with type 1 diabetes. At present, the number of patients becoming insulin-independent is rapidly increasing world-wide applying the transplantation protocol originally described by the group in Edmonton. A hallmark in this procedure is repeated infusions of islets obtained from 2 to 4 donors until normoglycemia is achieved. In order to establish islet transplantation as a widely accepted treatment modality, and make tolerance induction regimes applicable, it is essential that the donor:recipient ratio is brought down to 1:1. A conceivable strategy to achieve this goal in clinical islet transplantation is discussed.

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Islet transplantation has been explored as a treatment for patients with type 1 diabetes since the development of collagenase digestion of the pancreas (1, 2). Initially, it was expected that the technique for islet isolation that had been developed for the rodent pancreas could also be applied to the human pancreas with only small modifications. However, it was not until the development of an automated method for islet isolation and continuous density gradients for separating exocrine fragments and islets that the field moved forward in humans (3–6).

Insulin independence after islet transplantation in a patient with type 1 diabetes mellitus (T1DM) was first achieved 10 years ago (7). However, after only 22 days, insulin treatment had to be reinstated. The cause of this graft failure has been debated, but since then most patients transplanted with islets isolated from one pancreatic gland have experienced no, or only short-term, freedom from exogenous insulin injections, irrespective of the immunosuppressive regimen applied. In contrast to these rather disappointing results, islet auto-transplantation studies in patients who otherwise would have suffered with non-autoimmune diabetes following surgical pancreatectomy indicated by chronic pancreatitis have demonstrated that the procedure can result in long-term insulin independence in these patients (8, 9). The reasons for the difference in outcome in the two groups of patients are unknown, but both the lack of peripheral insulin resistance and allo- and autoimmunity have been advocated as tentative causes.

Alternative approaches have been explored over the years by many including the research group in Edmonton, Canada, who have hypothesized that more islets than can be isolated from a single pancreas are needed in order to achieve insulin independence in patients with T1DM. Their strategy has been developed in parallel with the application of refined immunosuppressive regimes. Initially, induction therapy consisted of high doses of steroids or ATG at the time of transplantation. A major limitation of these induction regimes was that they could only be maintained for a short period of time, during which the chance to obtain islets from a second pancreas was small. The approach originally taken by the Edmonton group to overcome this problem was to use frozen islets, which could be stored until needed. Applying this protocol long-term insulin independence was achieved in a patient with T1DM by transplanting freshly isolated islets from one pancreas, pooled together with freeze-thawed islets from an additional 10 pancreases (10). This approach was, however, hampered by problems in maintaining satisfactory quality and quantity of the freeze-thawed islets.

The subsequent development of new immunosuppressive drugs allowed the induction therapy to be extended from a week to several months. In an already classical report, the Edmonton group utilized this opportunity to repeat the islet transplantation procedure, using freshly isolated islets from 2 to 4 donors, until the patients were able to maintain normoglycemia without exogenous insulin administration (11). The procedure can be regarded as an islet dose-finding-study in persons with T1DM, and it was concluded that a total of about 12,000 islet equivalents (IEQ)/kg BW were needed to achieve insulin independence in humans.

In a follow-up study of these patients, the functional capacity of the transplanted islets was estimated to correspond to only about 20%–40% of that in a non-diabetic person (12, 13). Together, these reports strongly suggest that only a small fraction of the transplanted islets successfully
engraft. In experimental islet transplantation it is generally accepted that 50%–70% of the transplanted islets will be lost in the immediate posttransplantation period (14, 15). Since glucocorticoids were not used in the Edmonton study, and thus could not have exerted any adverse effects on islet function, the authors emphasized that other adverse processes must be involved in the loss of islet tissue (11).

We and others have demonstrated in a series of reports that a thrombotic/inflammatory reaction is elicited when islets come in direct contact with ABO-compatible blood (16–18); this reaction is characterized by a rapid binding to and activation of platelets on the islet surface, as well as activation of the coagulation and complement systems. Within 15 min, leukocytes are seen to infiltrate the islets. After an hour, most of the islets are infiltrated by numerous leukocytes (both monocytes and granulocytes), resulting in disruption of islet integrity and islet loss. This reaction is termed the instant blood-mediated inflammatory reaction (IBMIR) and is induced by inflammatory mediators (such as tissue factor (TF) and MCP-1) that are expressed by isolated human islets. In clinical islet transplantation, IBMIR is triggered within minutes after islet infusion into the portal vein (17). Taken together, these findings indicate that IBMIR is induced as a consequence of the interplay between the transplanted islets and the host. The detrimental effects of IBMIR provide an explanation for the relatively low success rate of clinical islet transplantation and the host. The detrimental effects of IBMIR are seen to infiltrate the islets. After an hour, most of the islets are infiltrated by numerous leukocytes (both monocytes and granulocytes), resulting in disruption of islet integrity and islet loss. This reaction is termed the instant blood-mediated inflammatory reaction (IBMIR) and is induced by inflammatory mediators (such as tissue factor (TF) and MCP-1) that are expressed by isolated human islets.

THE NUMBER OF ISLETS IN A PANCREAS REVISITED

The number of islets in a human pancreas has been determined using thorough morphometrical analysis. In a study involving seven pancreases, the total number of islets varied between 3.6 and 14.8 million (19), and the total islet volume was 0.5 to 1.3 cm³. In that study all islets were included, and a large number of small islets dominated the reported value. Bo Hellman had previously reported similar findings in a series of publications (20, 21). From the figures reported, the total number of islets with a diameter of ≥23 μm in a human pancreas could be estimated to be about 1.5 million. Volumetric distribution analysis showed a maximum corresponding to islets with a diameter of about 140 μm, with a remarkable degree of regularity between the pancreases examined. The average percentage (v/v) of islets in the pancreas was 1.3%, with a progressive increase from the caput to the cauda. These data were used to construct Table 1, showing the distribution of islets and their relative contribution to the total islet volume in a normal pancreas. Islets with a diameter of 100 μm or more constituted about 20% of the total number of islets but about 75% of the total islet volume.

When the presently available technology for islet isolation is used, most of the small-sized islets are lost. There is also a considerable risk that a large fraction of the largest islets will be damaged during the isolation procedure. However, even if only the medium-sized islets are harvested, only a small fraction of the total islet volume is lost. This finding is in accord with reports from most centers with an active clinical islet transplantation program; most centers report that they succeed in obtaining 300,000–600,000 IEQ/pancreas, constituting about 4 and 10 cm³ in volume. Theoretically, about 500,000 islets with a diameter of 150 μm (the diameter of the standardized islet; IEQ) comprise the total islet volume in a normal 70-g pancreas (≈1 cm³).

This notion that a major fraction of the total islet volume is successfully retrieved after islet isolation is further substantiated in a study in which the total insulin content in the native pancreas was determined and compared with that in the final islet preparation after purification. In none of the 20 isolations included in the study was a decrease in total insulin content found, although there were differences in the total insulin content between individual pancreases (22).

Altogether, it seems as if the presently applied technology for islet isolation enables an experienced investigator to obtain a major fraction of the total islet volume in a pancreas of good quality. Consequently, future improvements in the islet isolation procedure may result in only a limited increase in islet volume but should focus on improving islet quality. Over the last decade, several studies have reported an increase in islet yield, from about 250,000 IEQ to about 500,000 IEQ in the experimental group (23–30). Hence, the number of islets isolated from a pancreas seems to be limited to about 500,000 IEQ, close to the theoretical number of IEQ in a pancreas.

ISLET GROWTH AFTER TRANSPLANTATION

B-cell replication has been thoroughly examined in experimental studies using rodent as well as human islets. Although B-cell replication has been found, almost no study has shown an increase in islet volume in vitro or after transplantation. Indeed, most studies have reported a decrease in total islet volume after transplantation when compared to the volume implanted. Corresponding data for clinical islet transplantation are not yet available. However, results of hemipancreatectomy and transplantation of the pancreas segment have indicated that normoglycemia (indicated by normalized hemoglobin A1c) can be maintained with only 50% of the normal islet volume for up to 18 years after transplantation (31). Although some of the donors and recipients developed overt diabetes, this correlated with the development of ob-

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Calculations are based on results presented in (references 18, 19) and reflect the situation in a normal human pancreas with about 1.5 million islets with a diameter of 23 μm or more and a total islet volume of about 1 mL (≈about 500,000 IEQ, 0.150 μm).

Notably, the total number of islets with a diameter of 100 μm or more constitutes almost 80% of the total islet volume, but only 20% of the total number in the pancreas.
sity. However, detailed analysis revealed an impaired functional capacity even in the group of normoglycemic individuals. Several of the donors were found to have an impaired response to an oral glucose tolerance test when examined 60 months after donation. The increased secretory demand on the remaining B cells in these hemi-pancreatectomized donors is evidenced by the development of hyperproinsulinemia after the procedure (32). Two important conclusions relevant for the present discussion can be drawn from these studies: (i) the reserve capacity of islets in the human pancreas is about 50%, and (ii) there is no, or only limited, compensatory increase in islet volume even many years after resection or transplantation. Limited islet growth has also been observed in a follow-up study of patients receiving transplants as part of the Edmonton series, in whom the functional capacity of the transplanted islets was estimated to be only about 20%–40% of that in non-diabetic persons (12, 13).

**ISLET SURVIVAL RATE AFTER INTRAPORTAL TRANSPLANTATION**

Based on the results presented above, the frequency of islets surviving the transplantation can be calculated. This calculation is based on the following: (i) that the average number of IEQ in a normal-sized pancreas is about 500,000; (ii) given normal islet function after islet transplantation, the number of surviving islets in the Edmonton patients is 20%–40% of that in a normal pancreas; (iii) no growth or only limited islet growth occurs after transplantation; and (iv) the patients receive about 12,000 islets/kg, corresponding to about 850,000 IEQ transplanted into a person with a body weight of 70 kg.

Based on these assumptions, the survival rate of the implanted human islets can be estimated to be only about 10%–20%. This figure may seem low, but it is only marginally lower than that reported after transplantation of rodent and human islets in experimental studies (14, 15). It should, however, be remembered that the quality of the rodent islets, isolated immediately after retrieval of the pancreas from a young healthy donor, is better and far more standardized than that of human islets.

The apparent difference in success rates between autologous and allogeneic transplantation may also be due to donor-related circumstances. The number of islets needed to achieve normoglycemia in patients receiving an islet autotransplant is far less than that needed in allogeneic transplantation (33). Similarly, insulin independence is regularly achieved with islets obtained from only one pancreas in primate models of islet transplantation (34–37). In these instances, just as in the rodent studies, the donor pancreas has not been subjected to the disrupted physiology that accompanies brain death while islets used for clinical allogeneic transplantation come from brain-dead donor pancreata. This means that the allogeneic pancreas is often obtained from a donor who has been subjected to intensive care for a prolonged period of time (as long as several days). This situation imposes considerable stress on the donor and the presence of acute brain injury in the donor is associated with release of proinflammatory mediators (38, 39). Furthermore, the islet tissue is also subjected to the procurement procedure, cold ischemia, and the islet isolation and culture procedures. Important stress factors throughout this series of events are hyperglycemia and hypoxia. In particular, hypoxia is a well-known trigger of inflammation, of which tissue factor (TF) expression is only one parameter. We have found and reported considerable variation in the amount of TF expression in islets obtained from different donors (17), and this suggests that expression may be induced. It also suggests that TF expression is only one feature in an array of proinflammatory events, including expression of MCP-1 (40, 41), IL-8, and MIF (42), that are triggered in the islets by the chain of events occurring before transplantation.

**THE LINK BETWEEN IBMIR AND THE SPECIFIC IMMUNE RESPONSE**

The development of immunosuppressive protocols that are able to induce immunological tolerance is critical to the long-term success of clinical islet transplantation. Achieving this goal would make islet transplantation an attractive therapeutic option for people with T1DM at an earlier stage, before late complications have started to appear. However, in addition to the direct damage that IBMIR inflicts on the infused islets, it also provides a powerful “danger signal” to enhance antigen presentation, leading to an accelerated and reinforced T and B cell-mediated response (analogous to the adjuvant effect in immunization/vaccination).

Given the new insight that islets infused in the portal vein regularly induce IBMIR, even in the absence of clear clinical signs, it is apparent that the problem created by IBMIR must be overcome before any meaningful attempts to induce tolerance at the clinical level can be initiated. There are two reasons for this requirement: (i) the loss of islets due to IBMIR, and the important consequence that islets from several donors are needed to achieve normoglycemia; (ii) the strong “danger” signal that IBMIR sends to the specific immune response (i.e., rejection and autoimmunity).

These challenges may explain why the results of islet transplantation in patients with T1DM, in which the transplantation occurs across both an autoimmune and an allogeneic barrier, are inferior to those seen in patients who have undergone autologous islet transplantation, when no such barriers exist. It is noteworthy that none of the non-human primate models of islet transplantation include an autoimmune barrier, making it difficult to translate the results obtained in these animals to the clinical situation.

These hurdles in T1DM can only be controlled once we are able to overcome IBMIR. Therefore, control of this reaction is the first step toward clinical tolerance in islet allotransplantation, and without control of IBMIR, we believe the possibility of success for any tolerance protocol is very limited.

**HLA IMMUNIZATION AFTER ISLET TRANSPLANTATION**

A special concern in applying the Edmonton protocol for islet transplantation to a group of otherwise healthy patients with T1DM is the risk of HLA immunization. Currently, no HLA matching between donors and recipients is performed. Therefore, the individual patient may be exposed to...
CONCLUDING REMARKS

Even with current state-of-the-art techniques, several hurdles must still be overcome before clinical islet transplantation can be established as a therapeutic alternative for the treatment of patients with T1DM. It is essential that the donor:recipient ratio be reliably reduced to 1:1. Based on the results discussed herein, only limited improvements in the number of islets obtained from a pancreas can be expected. The focus should instead be redirected to minimize islet loss in the immediate posttransplantation period. Even a relatively small reduction in islet loss will have a major impact on the clinical outcome.

REFERENCES