Monitoring of the islet graft

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SUMMARY

The Edmonton trials have brought about a marked improvement in the short-term rate of success of islet transplantation with rates of insulin-independence of 80% at 1-year being reported by several institutions worldwide. Unfortunately, this rate consistently decreases to 10-15% by 5 years post-transplantation. Several mechanisms have been proposed to explain this apparent 'islet exhaustion', but are difficult to pinpoint in a given patient. Understanding the reasons for islet graft exhaustion and its kinetics is a prerequisite for the improvement of islet transplantation outcome. In this regard, efficient monitoring tools for the islet graft have been conspicuously lacking and are required to detect islet damage and diagnose its mechanisms in a timely fashion, so as to initiate salvage therapy such as antirejection treatment. Tools for the monitoring of the islet graft include follow-up of metabolic function but mostly indicate dysfunction when it is too late to take action. Progress is likely to arise in the fields of immune monitoring, molecular monitoring and islet imaging, notably thanks to magnetic resonance (MR) or positron emission tomography (PET) technologies.

Key-words: Islet of Langerhans transplantation • Monitoring • Imaging • Magnetic resonance • Positron emission tomography • Review.

RÉSUMÉ

Surveillance des greffons d’îlots de Langerhans

Les essais d’Edmonton ont amené une amélioration spectaculaire dans le taux de succès à court terme de la greffe d’îlots de Langerhans, avec des taux d’insulino-indépendance de 80% à 1 an rapportés par plusieurs institutions à travers le monde. Malheureusement, ce taux décroit de façon reproductible à 10-15% à 5 ans de la transplantation. Plusieurs mécanismes ont été proposés pour expliquer cet apparent “épuisement des îlots”, mais il est difficile de déterminer avec précision lequel est responsable de la perte de fonction chez un patient donné. La compréhension des raisons de cet épuisement et de leur cinétique est une condition préalable pour l’amélioration des résultats à long terme de la greffe d’îlots. À ce propos, nous souffrons d’un manque criant de moyens de monitoring des greffons d’îlots, qui permettrait pourtant de détecter une atteinte des îlots greffés et d’en diagnostiquer la cause à temps pour pouvoir débuter une thérapie de sauvetage appropriée, tel qu’un traitement anti-rejet par exemple. Parmi les outils de monitoring des greffons d’îlots figure le suivi métabolique, qui, en général, indique les signes de dysfonction du greffon trop tardivement pour pouvoir réagir efficacement. De grands progrès sont attendus dans les domaines du monitoring immunologique, du monitoring moléculaire et de l’imagerie des îlots, notamment grâce aux techniques de résonance magnétique et de tomographie par émission de positrons.

Mots-clés : Transplantation d’îlots de Langerhans • Monitoring • Imagerie • Résonance magnétique • Tomographie par émission de positrons • Revue générale.
Introduction

Islet of Langerhans transplantation has come to the forefront as one of the most promising approaches in the quest for a cure for type 1 diabetes. This is largely the result of the “Edmonton protocol”, that allowed for the first time consistent achievement of insulin independence after islet transplantation, thanks to a steroid-free immunosuppressive combination, and to sequential islet infusions in order to increase the transplanted islet mass [1]. Unfortunately, figures of 80% insulin independence at 1 year have not been sustained, and the latest update of the Edmonton experience reported insulin independence rates of approximately 10-15% at 5 years, although graft function (C-peptide positivity) was retained in a vast majority of patients [2]. Several phenomena are likely to be involved in late islet graft loss, such as allogeneic rejection [2, 3], recurrence of autoimmunity [3, 4], islet toxicity of the immunosuppressive (IS) drugs [5-8], lack of beta cell regeneration due to the antiproliferative properties of sirolimus, an IS drug on which the Edmonton protocol is based, or “exhaustion” of the islet graft [9].

These alleged mechanisms of islet graft loss are not mutually exclusive, and occur on a terrain of suboptimal beta cell functional reserve, as suggested by markedly decreased insulin responses to stimulation in islet transplant recipients as compared to controls [10]. The low engrafted islet mass is undoubtedly the major factor explaining the fact that reversal of diabetes with islets isolated from a single donor is very uncommon [11]. This is thought to arise as a result of: early islet loss during the isolation procedure or in the graft microenvironment within the liver, ischemia-reperfusion-like injury and non-specific inflammatory phenomena [12-17]. An acute inflammatory process that instantly destroys a large part of islets injected intraportally upon contact with blood was described recently, and is thought to be a major determinant of early islet graft loss [16, 17].

There is undoubtedly a lot of room for improvement in the long-term and short-term survivals of islet graft, but this will only be achieved when mechanisms of islet destruction have been better understood and characterized. In this regard, the whole field of islet transplantation suffers from a blatant lack of monitoring tools able to detect graft damage or decrease in graft mass or function in a timely manner. Therefore, techniques of islet graft monitoring that will be developed must address the three following aims: (i) understanding when islet grafts are damaged; (ii) understanding by which mechanism(s) islet grafts are damaged; (iii) detecting islet damage early enough to allow for appropriate intervention to salvage the graft.

Metabolic monitoring

Current clinical monitoring is based on metabolic islet function and utilises serum markers, in the basal and stimulated states [18]. These markers, recapitulated in table I, have been used for decades in the baseline assessment of the diabetic patient and need not be discussed in detail here. They are recorded at each visit and based on them, islet grafts can be classified as being fully (insulin-independence), partially (insulin required and detectable C-peptide) or not functioning (no detectable C-peptide). None of these markers are specific of the islet transplant situation and can be used in the assessment of any diabetic patient.

Islet graft function

Measuring C-peptide levels is the simplest way of assessing islet function in a subject receiving exogenous insulin. Because C-peptide levels vary a lot according to blood glucose, they have hardly any quantitative value. Moreover, islet recipients have various degrees of impaired kidney function, which impacts on C-peptide excretion and prolongs its serum half-life.

The Secretory Unit of Islet Transplant Objects (SUITO) was recently developed. It computes both blood glucose and C-peptide and can be calculated with the following formula: (1500*fasting C-peptide [ng/dl]) / (fasting blood glucose [mg/dl]-63). The SUITO index allegedly represents accurately the percentage of a “normal” islet mass in a given subject but still needs validation [19]. However, none of these methods accurately quantifies islet function, because they do not take the quality of metabolic control into account.

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**Table I**

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<th>Metabolic monitoring of the islet graft.</th>
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<td>Overall function</td>
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<td>Glucose stability</td>
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<td>Stimulation tests</td>
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The Beta score was introduced to take all these parameters into account. It is based on fasting blood glucose, HbA1c, daily insulin requirements, use of oral hypoglycaemic agent and stimulated C-peptide [20]. It rates islet graft function on a 0-8 scale, with 0 corresponding to total absence of function and 8 to perfect graft function (table II).

Metabolic stability

Monitoring can be refined by quantifying blood glucose instability [21]. The MAGE (Mean Amplitude of Glycaemic Excursions) index reflects blood glucose stability. It can be calculated from 14 consecutive blood glucose values taken over 48 hours at pre-defined time points, by calculating the arithmetic mean of blood glucose increases or decreases, that exceed one standard deviation of the blood glucose values measured during the study period. Control individuals have a MAGE index comprised between 1 and 3.3 mmol/l, while patients with unstable Type 1 diabetes can have values up to 15 mmol/l [22, 23]. The MAGE index is easy to calculate, can be repeated often and has been broadly used to assess metabolic stability in islet transplant recipients [1, 24, 25].

The Lability Index (LI) also reflects blood glucose stability. It was tested on a large group of islet transplant patients and was found to correlate better with the clinical assessment of lability than the MAGE index. However, it is a cumbersome index to calculate and requires several values measured over four weeks [26]. The following sum has to be computed for each one of four consecutive weeks: \( \sum ((\text{Gluc}_n - \text{Gluc}_{n+1})/h_{n+1} - h_n) \), where “Gluc” (in mmol/l) is the nth reading (rounded to the nearest hour). The LI can then be calculated as the mean value of this sum over the four weeks. Most patients with type 1 diabetes have a LI up to 400 mM²/h·week, and patients selected for islet transplantation for metabolic instability have a LI up to 700 mM²/h·week. To our knowledge, the LI has not been tested on a large group of islet transplant patients and was found to correlate better with the clinical assessment of lability than the MAGE index. However, it is a cumbersome index to calculate and requires several values measured over four weeks [26].

Blood glucose stability and the occurrence of hypoglycaemia can be measured very accurately using the Continuous Glucose Monitoring System (CGMS). It requires the placement of a subcutaneous probe, which is removed at the end of the recording. The device measures capillary glucose levels continuously over a few consecutive days. Several groups have used this technology [27-29] for the monitoring of islet grafts.

Stimulation tests

While the tests described above provide a snapshot idea of islet function or glucose stability at a given time or period, they give no information on islet response to a standardized stimulus. Stimulation tests answer this question and can be classified in two groups, depending on whether they study islet response to a glucose challenge or to direct pharmacologic stimulation. All stimulation tests should be performed in the fasting state and after discontinuation of exogenous insulin for at least 12 hours, which makes them impractical to perform in islet transplant recipients who are still on insulin.

The mixed-meal tolerance test (MTT) provides simple information about islet function. Blood glucose, C-peptide and insulin levels are measured prior to and 90 minutes after ingestion of a standardized meal, usually a commercial liquid caloric supplement. Differences in cross-border availability of these products make standardization of the meal only relative. Study subjects ingest a preparation containing 350-500 kcal and 50-65 g carbohydrates depending on product used. A normal response to the MTT in control subjects shows a stimulated C-peptide level of 1000 to 1500 pmol/l.

In the oral glucose tolerance test (OGTT), blood sugar is measured after ingestion of 75 g of oral glucose, with blood samples drawn at 30, 60, 90 and 120 min. The OGTT is the only metabolic stimulation test included in the American Diabetes Association (ADA) definition of impaired glucose tolerance and diabetes [30].

In the intravenous glucose tolerance test (IVGTT) 300 mg/kg body weight of 50% dextrose is perfused intravenously over 1 minute after two baseline samples (-10 and 0 minutes) for glucose, insulin and C-peptide were drawn. Sampling is then usually done at 3, 4, 5, 7, 10, 20, 30, 40 and 50 minutes after glucose infusion [31]. This test allows the calculation of the acute insulin response to glucose based on the mean of the insulin level at 3, 4 and 5 minutes minus the mean basal insulin level at -10 and 0 minutes. Glucose disposal rate (\( K_g \)) is calculated as the slope of the natural logarithm of

Table II

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<th>Score</th>
<th>2</th>
<th>1</th>
<th>0</th>
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<tbody>
<tr>
<td>Blood glucose [mmol/l]</td>
<td>≤ 5.5</td>
<td>5.6-6.9</td>
<td>≥ 7</td>
</tr>
<tr>
<td>HbA1c [%]</td>
<td>≤ 6.1</td>
<td>6.2-6.9</td>
<td>≥ 7</td>
</tr>
<tr>
<td>Daily insulin [units/kg] or OHA</td>
<td>none</td>
<td>0.01-0.24 and/or OHA</td>
<td>≥ 0.25</td>
</tr>
<tr>
<td>Stimulated C-peptide [nmol/l]</td>
<td>≥ 0.3</td>
<td>0.1-0.29</td>
<td>&lt; 0.1</td>
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1. Assessment of islet graft function, according to Ryan et al [20].
2. OHA (oral hypoglycaemic agent)
the glucose values. It may reflect endocrine reserve as well as insulin resistance. KG values < -1.0 are considered normal. Areas under the curve (AUC) for insulin and C-peptide can also be calculated.

The arginine-stimulation test [32] is gaining high interest in the follow-up of islet transplant recipients, because it is easy to administer, devoid of significant side-effects and does not involve a carbohydrate challenge and ensuing hyperglycaemia. Serum insulin is usually measured –10, 0, 2, 3, 4, 5, 7 and 10 min from intravenous injection of 5 g arginine over 30 sec [25]. The AUC for insulin reflects islet mass. The acute insulin response (AIR) can be calculated as the mean of the three highest values between 2 and 5 minutes minus the mean of values at –10 and 0 minutes. In healthy volunteers, mean AUC was 183 ± 57 mU/min/l and mean AIR 31.5 ± 9.5 mU/l, values that are hardly achieved by recipients of successful islet transplants, indicating suboptimal engrafted islet mass [25]. We have recently reported that follow-up of the AIR in arginine stimulation tests was a good prognostic indicator of islet graft outcome and that a decline in AIR always occurred before reintroduction of exogenous insulin [33].

In many institutions, the arginine stimulation test has replaced the glucagon-stimulation test (insulin secretion after intravenous injection of 1 mg glucagon), which is linked to side-effects, such as nausea and vomiting and only provides semi-quantitative information about islet responsiveness in terms of insulin and C-peptide release in a stimulated condition [34].

The principal problem with metabolic tests (hyperglycaemia, loss of C-peptide, absence of response to stimulation, etc.) is that they are late markers of islet graft dysfunction, and generally appear when it is no longer possible to salvage a failing graft. For this reason, development of novel techniques of islet graft monitoring or identification of surrogate markers of rejection is of foremost importance.

**Monitoring of rejection**

Although it is far from certain that it is a significant cause of long-term islet graft loss, islets of Langerhans are prone to acute rejection. Chronic rejection of islet grafts has not been characterized and it is as yet unclear whether it could be a cause of progressive islet graft exhaustion and associated decrease of function. Therefore, it is obvious that islet of Langerhans transplants should be followed-up for rejection as closely as other organs. Unfortunately, there is currently a lack of efficient tools to monitor islet rejection, and intense research is taking place in order to develop accurate methods of diagnosis or identify surrogate markers of islet graft rejection.

**Islet graft biopsy**

In all solid organs, histological examination of the graft is the gold standard diagnostic test for acute rejection. However, a biopsy is an invasive procedure and complications may occur. Moreover, in the case of islet transplantation, the quantity of islets engrafted in the liver is very low. Considering that the total volume of 1 million islet equivalents is approximately 1.8 ml and that the volume of the liver corresponds to 1.5-2% of the total body weight (i.e. 1,000-1,500 ml in a 70 kg subject), a large islet graft will occupy 0.1-0.2% of the total liver volume. It is then easy to understand that percutaneous needle biopsies have low chances of sampling islets, unless multiple biopsies are performed. Therefore, liver biopsies have not entered clinical routine yet, although some centers have undergone such activity [9]. In order to have a more accessible site for biopsy, it has been proposed to transplant some of the islets in the forearm, as a sentinel graft [35]. Although the idea looks appealing, the method is limited by the fact that the implantation and the survival of islets are probably site-dependent, and islets implanted at two different sites are unlikely to behave in a similar fashion.

In the specific case of combined organ transplant, such as simultaneous islet-kidney transplantation, rejection is known to usually occur on both organs at the same time. Recipients of combined islet/kidney transplants will thus be treated whenever rejection is detected in the kidney.

**Detection of humoral anti-HLA reactivity**

Humoral responses against donor HLA molecules can be as deleterious to transplanted organs as cellular rejection [36]. There have been reports of the detection of circulating anti-HLA antibodies specifically directed against donor antigens and preceding the occurrence of islet graft failure [37-39]. Although these observations suggest that humoral responses might have played a role in the failure of the islet grafts, they do not provide absolute demonstration that humoral rejection indeed occurred. Nonetheless, detection of circulating anti-HLA antibodies at regular intervals during follow-up seems valuable and has entered the routine of islet transplantation programs [25]. Detection of anti-HLA antibodies can be done using several techniques with different sensitivities. The method of the panel-reactive antibodies (PRA) assesses anti-HLA reactivity by measuring the percentage of cells from a panel of blood donors against which the recipient’s serum reacts using a complement-dependent cytotoxicity assay. The classic PRA method was improved with the introduction of the techniques of enzyme-linked immunosorbent assay (ELISA) and more recently of flow cytometry. Both methods utilize purified class I and class II HLA antigens as targets for the binding of anti-HLA antibodies from the patient’s serum. HLA antigens are coated on the assay plate for the ELISA method or on polystyrene beads for flow cytometry. In comparison to the cytotoxicity PRA assay, these new methods are associated with higher sensitivity, especially for the detection of anti-class II antibodies, and allow determination of alloantibody specificity [40, 41].

**Detection of cellular anti-HLA reactivity**

The detection of cellular anti-HLA responses is more difficult and is not performed routinely, because it implies the
realisation of labor-intensive and complex in vitro assays. The cytotoxic T-lymphocyte precursors (CTLp) and the helper T-lymphocyte precursors (HTTp) assays measure cytotoxicity and IL-2 or other cytokines production in limiting dilution assays, where decreasing numbers of recipient donor lymphocytes are incubated with a fixed number of irradiated donor-specific stimulator cells (or cells with any desired number of HLA matches or mismatches with respect to the donor and/or the recipient). Conflicting data have been reported on the usefulness of these assays in predicting solid organ rejection [42]. To our knowledge, the CTLp assay has been performed by one group on peripheral blood lymphocytes of islet transplant recipients, who were found to exhibit absent or low responses, except in one patient in whom strong responsiveness correlated with islet graft failure [43].

Tetramer technology has revolutionized the field of detection of antigen-specific T-cells. It consists of 4 biotinylated MHC molecules covalently linked together by streptavidin, thus increasing their affinity to the T-cell receptor during cognate interaction. Class I or class II HLA-peptide tetrameric complexes allow direct ex vivo visualization of antigen-specific CD8+ or CD4+ T-cells in straightforward, easy to perform assays [42, 44]. Tetramer technology has yet to be made available for the quantification of specific anti-HLA T-cells, but would be a welcome tool for the assessment of donor-specific cellular reactivity.

Finally, it should be briefly mentioned that immune monitoring is currently generating a lot of interest in the transplantation community. New methods for the ex vivo and in vitro measurement of immune events of clinical significance in a transplant recipient are being developed, with the aim to detect states not only of rejection, but also of tolerance (or rather low responsiveness) that might allow tapering of immunosuppression [42, 44]. Detection of lymphocyte gene transcripts is one method of immune monitoring currently under development and will be discussed below.

**Monitoring of autoimmunity**

Islet grafts are prone not only to destruction by allogeneic rejection, but also by recurrence of autoimmunity [3]. Recurrence of autoimmunity in transplanted islet tissue was clearly demonstrated by the observation of graft failure caused by insulinis in recipients of segmental pancreatic grafts from an identical twin. These patients had received no immunosuppression because there was no risk of rejection [45].

**Autoantibodies**

In islet transplant recipients, there is indirect evidence that autoimmunity participates to graft failure in spite of adequate conventional immunosuppression. This was first suggested when the Giessen group reported that islet graft failure occurred significantly earlier in patients testing positive for the presence of islet cell antibodies (ICA) or anti-GAD65 autoantibodies [46]. This observation was confirmed by the Milan group [47], who also reported that a rise in autoantibody titers in recipients of vascularised pancreas transplants was observed in a minority of patients (7%), but almost invariably followed by graft function failure [48]. Kinetics of autoantibody titers show great patient-to-patient variability and clear guidelines regarding action to be taken in the situation of a rise of autoantibody levels are still lacking. Nonetheless, it seems valuable to measure autoantibody titers in the follow-up of an islet transplant recipient, as a marker of autoimmunity reactivation. A marked rise in titers might prompt the investigator to perform a liver biopsy looking for insulinis or even to administer immunosuppression in the same way one would treat a bout of acute rejection.

Autoantibodies of interest comprise ICAs, anti-insulin autoantibodies (IAA), anti-glutamate decarboxylase 65 (GAD65) autoantibodies, and anti tyrosine phosphatase (IA-2) autoantibodies. Levels of ICAs are determined by indirect immunofluorescence on frozen sections of human pancreas, whereas IAAs, GAD65 and IA-2 antibodies are determined by radio-immunooassay.

**Autoreactive T-cells**

GAD65 and IA-2 are probably the major autoantigens in type 1 diabetes, and elicit autoantibodies that are the most specific markers of the disease. However, beta-cell destruction in type 1 diabetes is primarily, if not only, T-cell mediated, and it is unlikely that autoantibodies are directly pathogenic [49]. Therefore it seems logical to attempt to monitor directly the presence of autoreactive T-cells in the peripheral blood of islet transplant recipients in order to detect recurrence of autoimmunity. The tetramer technology briefly described above has been used to characterize autoimmune responses in patients with Type 1 diabetes ant at-risk subjects [50]. HLA-DR tetramers containing a peptide corresponding to the immunodominant epitope GAD65(555-567) from human GAD65 were first used for that purpose, and CD4+ T-cells were detected by flow cytometry in the blood of all Type 1 diabetic patients, some at-risk subjects but no control subject [50]. Of great significance, is the recent report of the detection of CD8+ autoreactive T-cells in the peripheral blood of patients with type 1 diabetes, using HLA-A2 tetramers binding an insulin peptide (insB10-18) and the observation that their occurrence in islet transplant recipients was strongly associated with graft failure [51]. Autoreactive T-cell monitoring using the tetramer technology is undoubtedly going to be very shortly an important tool in the follow-up of islet transplant recipients.

**Molecular monitoring**

**Insulin gene expression**

We have recently reported the detection by reverse transcriptase-polymerase chain reaction (RT-PCR) of circulating insulin mRNA in the peripheral blood early after islet transplant recipients.
transplantation, and proposed that this apparent release of beta cell material could be a reflection of early islet damage in the engraftment period, with ensuing release of beta cell material in the peripheral blood [52]. This was comforted by the observation that circulating insulin mRNA was detectable for a much longer time (up to 10 weeks) in patients on a steroid-containing immunosuppressive regimen known to be toxic to the islets than in patients on a steroid-free regimen (up to 2 weeks).

In a subsequent study, we tested the hypothesis that monitoring of circulating insulin mRNA could be a valuable tool for the prediction of injury to the islet graft, in an attempt to identify a surrogate marker of acute islet rejection or recurrence of autoimmunity. In contrast to the previous study, we used a real time quantitative RT-PCR with the aim to correlate the amplitude of the mRNA peaks with signs of islet damage, and we followed-up patients for up to 500 days [53]. In this second study, insulin mRNA was always detected immediately after islet transplantation, and the duration and amplitude of the primary insulin mRNA peak was not correlated to graft size or outcome. Subsequent peaks of insulin mRNA were sometimes detected and were associated with alterations of islet graft function (increase in the amount of injected exogenous insulin, decrease in C-peptide levels, increase in HbA1c) when they were prolonged or of high amplitude. With appropriate cut-off values, positive and negative predictive values of 80% were obtained [53]. Signs of islet damage were observed on average 17 days after detection of the secondary peak, suggesting the usefulness of the assay for timely graft salvage. Interestingly, thanks to the use of quantitative RT-PCR, we were able to calculate that the average amount of insulin mRNA in the detected peaks was lower than the contents of one beta-cell, and that, in contrary to what we had previously stated, we were in all probability not detecting whole circulating beta-cells, but rather beta-cell material within phagocytes.

Our assay is in fact indicative of beta cell damage in general and is not specific for allorejection or recurrence of autoimmunity, two conditions that could be treated with a boost of immunosuppression. The fact that islets can also theoretically be damaged in the long term by non-specific inflammatory mechanisms or lose function to progressive exhaustion makes it necessary to couple it to another monitoring assay, so as not to initiate unnecessary antirejection treatment.

**Cytotoxic lymphocyte gene expression**

The T-cell-dependent immune activation gene products granzyme B, Fas-ligand and perforin have been involved in mechanisms of apoptotic death of target cells during the process of acute rejection. An increase of the levels of expression of these genes in the peripheral blood of kidney transplant recipients was shown to be associated with acute kidney graft rejection [54]. From this study came the idea of monitoring cytotoxic lymphocyte gene expression as a marker of rejection. In a first study on non-human primates from the Miami group, a sustained elevation of cytotoxic lymphocyte gene mRNA levels was observed 83-197 days before islet graft failure secondary to acute rejection [55]. Granzyme B was the best predictor of rejection. These findings were confirmed in a series of 13 patients, in whom a clear elevation of granzyme B mRNA levels was observed 25-203 days before onset of frequent hyperglycaemia and eventually re-introduction of insulin [56]. These markers of cytotoxic T-cell activity are likely to appear in the presence of an immune phenomenon such as rejection or recurrence of autoimmunity, but also in response to infectious or inflammatory processes, as reported in the study, accounting for a relative lack of specificity [56].

In this regard, coupling of our mRNA assay with the granzyme B assay might improve the specificity of both tests and enable to discriminate between immune and non-immune islet damage (granzyme B), and between immune islet destruction and infectious/inflammatory events (insulin). Such discriminating ability could provide an accurate trigger for the appropriate initiation of antirejection therapy.

**Islet graft imaging**

The field of beta cell imaging is currently generating a lot of interest, notably by the US National Institutes of Health, who have organized workshops on the theme of “Imaging of pancreatic beta cell in health and disease” and made funding available for clinical research projects on this topic. While the field encompasses the imaging of beta cells in type 1 or type 2 diabetes, emphasis was put on the need for imaging techniques of transplanted islets [57]. Imaging could be used as a tool to visualize either the islets directly to monitor graft mass, or an inflammatory process in a situation of ongoing acute rejection. Three modalities have demonstrated applicability in the near future: bioluminescence imaging (BLI), magnetic resonance imaging (MRI) and positron-emission tomography (PET).

**Bioluminescence imaging**

BLI uses light-generating enzymes such as luciferase, generating low-light signals easily detectable by exquisitely sensitive charged-couple device (CCD) cameras. Imaging islet transplants using this technology was reported in the mouse, with islets expressing the reporter gene luciferase, either by adenovirus-mediated gene transfer or transgene expression [58, 59]. BLI was shown to have a high sensitivity, being able to detect as few as 10 islets. Stable luminescence was obtained for as long as 18 months after transplantation [59]. Interestingly, luminescence intensity started to decrease several days before permanent recurrence of diabetes and histologically demonstrated acute rejection in an allogeneic model. Limitations for scaling up CCD technology results from the absorption of the BLI signal by mammalian tissue and ensuing penetration of only a few centimeters [59].
is much to be improved in this elegant method before it can be applied to large animals, let alone the human, notably the finding of a reporter gene with a much more favorable light emission spectrum for signal detection through mammalian tissue.

**Magnetic resonance imaging**

In contrast to BLI, MRI can be easily used in the clinical setting. The first attempts at liver MRI after islet transplantation were aimed at studying a possible structural impact on the liver of the procedure. The Philadelphia group discovered and reported the presence of several areas of focal steatosis around portal spaces in two patients, a finding that seemed to be more prominent in patients having good islet function [60]. It was assumed that this finding was the result of the paracrine action of local insulin release around implanted islets, but was probably of no clinical significance.

Further MRI studies used superparamagnetic iron oxide (SPIO) nanoparticles, which are widely used in the clinical setting as contrast agents for liver imaging. Two groups at the University of Prague, Czech Republic, and at the Massachusetts Institute of Technology have used such particles to label islets prior to transplantation in a rat model. After intraportal infusion, labeled-islets could be identified within the liver of rats and appeared as hypointense spots on $T_2^*$-weighted MR images. The signal remained stable within the liver and could allow imaging of islets for several months after syngeneic transplantation [61, 62]. In a model of allotransplantation without immunosuppression, no more MR signal could be detected three weeks after transplantation [63, 64]. Iron labeling did not affect islet viability, nor was a murine reporter gene transfected with SPIO particles by electroporation [65]. A possible improvement of the method was recently reported in the rat model, in which islets were transfected with SPIO nanoparticles routinely used for sequential monitoring of the islet mass in the long-term. This technique allowed detection of as few as 200 islets, but no data on its effect on islet function were provided.

Because of the availability of commercial, approved MR contrast agents made of SPIO nanoparticles routinely used for liver MR imaging, applying the method to the clinical setting is rather straightforward. We have recently included three islet transplant recipients in a pilot study, in which islets were incubated for 24 hours with ferucarbotran (Resovist®, Schering AG, Berlin, Germany) before intraportal transplantation. All three patients became insulin-independent, demonstrating the harmlessness of the iron-labelling procedure, and have shown hypointense spots on $T_2^*$-weighted sequences, as observed in the rat model, up to six months after transplantation so far [66]. This pilot study is a proof of principle of the validity of the concept and merely a starting point for the development of the method.

Analysis of the images should be improved. Iron particles induce a disturbance of the magnetic field, and the related image is larger than the particle itself. As a consequence, two similar spots can include various numbers of iron particles. 3-D reconstruction of the liver images in order to obtain a complete representation of hypointense spots and quantification of the implanted islet mass are the next steps. Further and most important, the clinical outcome has to be better correlated to the images. The critical point will be to know whether a decrease of signal can be detected early enough in order to be able to salvage a graft failing to rejection.

Other MR compatible contrast agents have also been studied. Lipophilic Gd³⁺ complexes, which bind to the cell membrane and are able to label islets ex vivo have been designed by Zheng et al [67]. Uptake of manganese, a MR enhancing agent, by glucose-activated beta cells has been observed and proposed as a method for functional islet graft imaging [68].

**Positron emission tomography imaging**

The sensitivity of PET is higher than that of MRI, and it allows accurate quantification of the signal. PET-compatible tracers can be used to label islets ex vivo, prior to transplant, or if specific enough, they can be injected intravenously after transplantation.

Ex vivo labeling of islet prior to intra-portal transplantation has been successfully attempted with 2-[¹⁸F]fluorodeoxy-D-glucose (FDG). Islets could be visualized for the first 6 hours after transplant only [69]. This study showed the feasibility of the technique in the context of islet transplantation, and was able to demonstrate that islets implanted only inside the liver. The same strategy was used with similar results in the pig [70]. Limiting factors for long-term assessment were the short half-life of the β⁺-emitting radionuclides (110 min for ¹⁸F) and the high outflow of tracer from the cell. However, this technique could have some indication in studying the fate of the islets very early after transplant.

PET imaging could also be performed after transplant for sequential monitoring of the islet mass in the long-term. This option would require the identification of tracers highly specific for beta-cells, but would also allow visualization of islets within the pancreas in patients suffering from type 1 or type 2 diabetes, or conditions such as insulinoma or nesidioblastosis. Considering the very low mass of islet grafts, it is expected that the probe should be retained at least 1000 times more by islets than by the surrounding tissue [71]. This is especially challenging considering that most tracers are metabolized by the liver, inducing a high background noise [72].

Beta cell-specific antibodies have been studied as potential tracking agents. The K14D10 monoclonal antibody and its Fab fragment (similar affinity, but faster clearance) were tested for that purpose. It was estimated that its cellular specificity was in fact too low to overcome the very low beta-cell mass in the pancreas [73]. An anti-IC2 monoclonal antibody (mAb) bound to a radioisotope chelator, showed decreased accumulation of the probe in streptozotocin-induced diabetic mice as compared to control animals. While analyses have been performed on native pancreas ex vivo, it is unclear whether such antibodies could be used for *in vivo*
clinical specificities for beta cells when tested in vitro [72]. [11C]Dihydrodopamine, nicotinamide and fluorodithizone had all low glibenclamide, tolbutamide, serotonine, L-DOPA, beta-cell specific radiolabelled mAbs.

While they were expected to be potential candidates, glibenclamide, tolbutamide, serotonin, L-DOPA, dopamine, nicotinamide and fluorodithizone had all low specificities for beta cells when tested in vitro [72]. [11C]Dihydrotetrazenzine (DTBZ) is a radio-ligand currently used in clinical imaging of the brain. It binds specifically to VMAT2, a transporter found specifically in the brain and in beta-cells. Longitudinal PET imaging of the native pancreas of diabetic BB rats demonstrated a decline of signal, paralleling the decrease of beta-cell mass [76]. This technique appears promising, but still needs to be replicated in the islet transplant setting in the context of the generally high uptake of the liver.

Conclusion

Efficient monitoring tools of the islet graft has been conspicuously lacking but are critically needed in the current era of high rates of short-term success and long-term loss of function experienced by the procedure. A wealth of candidate methods and techniques is close to hand and should allow significant progress in the understanding of the reasons for islet graft exhaustion and its kinetics, which is a prerequisite for the improvement of islet transplantation outcome. Progress is likely to arise from the fields of immune monitoring, molecular monitoring and islet imaging.

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