Published in final edited form as:

Curr Opin Organ Transplant. 2016 August ; 21(4): 412–418. doi:10.1097/MOT.0000000000000333.

Biomarkers In Pancreas Transplantation April 25, 2016

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Abstract

Purpose of review—This review analyzes the current biomarkers used in monitoring pancreas transplants (PT), from the simple and time-tested, to more sophisticated, including markers of allo- and auto-immunity, that are likely to play a larger role in future studies.

Recent findings—Evaluation of allo-immunity includes serum levels of Donor-Specific Antibody (DSA), and, ultimately, PT biopsies with C4d staining. Our center has focused on markers of auto-immunity, including assessment of autoantibodies and autoreactive T cells. We have found that conversion of autoantibodies (including GAD65, IA-2 and ZnT8), or the development of a new positive autoantibody, particularly ZnT8, are associated with recurrence of T1D (T1DR) in the PT. Autoreactive T cells have also been identified in the peripheral blood, PT and peri-PT lymph nodes, that have the potential to mediate human beta/islet cell destruction in vivo.

Summary—The monitoring of PT biomarkers, particularly those associated with autoimmunity, has lead to new insights into the pathogenesis of T1D. Progress in the elucidation of mechanisms of autoimmunity may lead to novel therapeutic approaches to both T1DR of the PT and perhaps also new onset T1D.

Keywords

Type 1 Diabetes; Pancreas Transplantation; Recurrent Autoimmunity; Autoantibodies; Autoreactive T cells

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Conflict of Interest: George W. Burke, III; Linda Chen; G. Ciancio; and Alberto Pugliese declare that they have no conflict of interest.

Introduction

Whole organ Pancreas Transplantation (PT) combined with kidney transplantation (KT), has become the therapy of choice for patients with type I diabetes (T1D) and end-stage renal disease (ESRD) (1). The PT restores euglycemia, without the need for exogenous insulin. The benefit of PT is particularly felt by those patients with long-standing T1D who reach the point of reduced hypoglycemia awareness. Recently, benefit has also been demonstrated in those patients with evidence of C-peptide secretion prior to transplantation i.e. patients with type 2 diabetes (T2D) (2,3). Since the PT is performed for its endocrine effect i.e. restoration of euglycemia/C-peptide secretion, patients are monitored for hemoglobin A1c (HbA1c), glucose and C-peptide levels. In addition pancreas exocrine markers, including serum levels of amylase and lipase, are followed since they offer further evidence of PT health and are often the harbingers of PT rejection, occurring prior to a detectable change in glycemic control. For those instances where the pancreas and duodenum are drained into the bladder, urine amylase may also be a useful marker of PT function.

Beyond the basic endocrine and exocrine markers of the PT there are important biomarkers that have begun to assume a larger role in the monitoring of PT function. These include markers of allo- and autoimmunity. The pancreas is relatively unique among solid organ transplants in that it is subject to both alloimmunity and autoimmunity. The alloimmune response to PT includes both cellular and humoral rejection. The autoimmune response involves recurrence of autoimmunity and is assessed by following levels of autoantibodies and autoreactive T cells. Ultimately for both allo- and autoimmune responses, a PT biopsy provides the necessary information for definitive diagnosis. Since restoration of glycemic control takes years to have a significant biological impact, as demonstrated in the DCCT study series (4,5) and David Sutherland's reports of PT effects on native kidney histology (6,7), evaluation of biomarkers that may prolong PT graft survival, is critically important to this field.

The Alloimmune Response

After revascularization of the PT, the blood glucose will begin to fall to normal levels in the operating room. The serum amylase and lipase will rise for the first 48 hours and then fall to normal values over the ensuing days as the PT recovers from the ischemia/reperfusion injury. In those cases of bladder drainage, the urine amylase will start to increase after 48 hours, measured as units of amylase per hour, and eventually peak in a range that is relatively consistent for each PT recipient. A subsequent rise in serum amylase/lipase, depending on how long after transplantation, will prompt an imaging study (ultrasound, or CT of the abdomen and pelvis) to rule out a surgical complication, e.g. duodenal leak. If there is no evidence of PT complication then acute rejection is considered likely. For bladder drained PT the urine amylase is assessed and typically the bladder is decompressed with a Foley catheter. If the serum amylase/lipase returns normal and the urine amylase returns to baseline (8), the hyper-amylasemia is ascribed to bladder distention which can be managed by adjusting voiding habits. Recently the monitoring of urine amylase to urine creatinine ratio has been demonstrated to be as effective as the measurement of urine amylase in terms of units per hour (8). If the urine amylase does not return to baseline, or for intestinal

drained pancreas transplants, if the serum amylase /lipase remain elevated, the PT recipient may be treated empirically with steroids and/or undergo a PT biopsy. For those PT with bladder drainage, if the urine amylase returns to baseline and the serum amylase/lipase return to normal after empiric steroid therapy then this is regarded as a steroid responsive, mild acute rejection episode. Otherwise the PT biopsy interpretation will direct the next level of therapy (9).

Cell mediated rejection seen on PT biopsy may be treated with steroids or for more severe rejection thymoglobulin. In the case of humoral or mixed cellular rejection, C4d is identified on biopsy and accompanied by DSA in the serum of the PT recipient. DSA may be de novo or persistent, if present from before the PT. While DSA is a hallmark of worse outcome in the in kidney transplantation (10), it is less clear in the context of PT (11). There are reports correlating DSA with PT graft failure but either de novo DSA was not distinguished from preformed consistently (12) or PT graft loss was not correlated with TP biopsy results (13). Thus DSA remains an attractive biomarker pre-and post PT, necessary to assess and to better understand the development of the alloimmune humoral response (11,14).

Autoimmunity Following PT

The first report of autoimmunity after PT was from Dr. David Sutherland when he performed living donor PT in twins or HLA identical siblings who received no or minimal immunosuppression (15,16). This observation lent support to the concept that T1D was an autoimmune disease. There have been other reports of recurrence of T1D (T1DR) identified in the explants of PT from deceased donors (17). However it is felt that immunosuppression sufficient to prevent rejection is also capable of preventing recurrence of autoimmunity (18) and so T1DR has been considered a relatively rare event. In Figure 1 we show an example of an islet from a PT of an SPK transplant recipient who developed T1DR. This is notable for the presence of a significant CD3 T cell infiltrate (insulitis), as well as insulin staining of residual islet/beta cells, although there is evidence of beta cell loss.

Identification of Autoantibodies and Autoreactive T cells in Patients with T1DR

We began studying T1DR in our simultaneous pancreas-kidney (SPK) transplant recipients in 2003 when we identified our first such patient. Most (95%) of our PT are performed in the context of SPK transplants, and nearly all involve bladder-drainage of the pancreas-duodenal transplant (1). This patient presented with severe, abrupt hyperglycemia (diabetic ketoacidosis – DKA) about five years after SPK transplantation, during which time the patient had been normoglycemic with no exogenous insulin required. The kidney function was unchanged/normal and urine amylase was stable and consistent. The patient underwent biopsy of both kidney and pancreas and was found to have normal kidney biopsy (no rejection), while the pancreas biopsy showed insulitis with CD3, CD4, CD8 and CD 20 infiltrates as well as the presence of insulin staining in the beta cells. We evaluated stored sera for levels of autoantibodies, specifically GAD65 and IA-2, and noted autoantibody conversion of both autoantibodies prior to developing hyperglycemia. GAD65-specific autoreactive T cells were also identified in the peripheral blood of this patient. In addition to

insulin staining present on PT biopsy, this patient also continued to have detectable C-peptide levels in the peripheral blood. In view of this it was felt that there was potential for therapeutic intervention and possible salvage of the PT function. This patient received a course of thymoglobulin and anti-CD 25 monoclonal antibody (daclizumab) with resultant transient increase in fasting C-peptide and loss of GAD65-specific autoreactive T cells from the peripheral blood. However a year later the GAD65-autoreactive T cells were again found in the peripheral blood. Subsequently the C-peptide levels fell, and PT function was lost (19).

Our experience in caring for patients with T1DR continued with a second patient who developed hyperglycemia 9 1/2 years after SPK transplantation. Hyperglycemia (DKA) occurred in the context of autoantibody conversion of both GAD65 and IA-2 autoantibodies. A pancreas transplant biopsy was performed on which insulinitis and the typical predominance of CD8 T cells were identified along with some degree of beta cell loss, however insulin staining was preserved in many islets. Autoreactive CD8 T cells were identified in the peripheral blood directed against the autoantigen IGRP. A combination of therapies including thymoglobulin, monoclonal anti-CD 25 antibody (daclizumab) and a single dose of rituximab were used. This resulted in reduction of autoreactive T cells below the level of detection that persisted for approximately one year. Initially fasting C-peptide levels increased after this treatment, however autoreactive T cells returned to the peripheral blood and C-peptide secretion was lost (19).

In most of our experience, patients who present hyperglycemic (DKA) after SPK transplantation still secrete residual amounts of C-peptide in their peripheral blood and insulin staining Beta cells are identifiable on the pancreas transplant biopsy (20). This allows us to justify treatment with immunotherapy in an attempt to preserve C-peptide secretion. However we have also identified a small number of patients who return with severe hyperglycemia after SPK transplantation in whom no C-peptide secretion is identified in the peripheral blood and on biopsy of the pancreas transplant, show no evidence of insulin staining. These patients have been treated with re-transplantation of the pancreas.

One specific example involves a patient who was diagnosed with hyperglycemia five years after SPK transplantation, who experienced conversion of autoantibodies including GAD65, and ZnT8 three months prior to clinical development of hyperglycemia. This patient received a second pancreas transplant. During retransplant surgery peri-pancreatic transplant tissue was resected in addition to a portion of the tail of the initial pancreas transplant for surgical reasons, specifically, enabling access to the external iliac artery and vein for perfusion of the second pancreas transplant. Autoreactive GAD65-specific CD4 T cells were identified in the peripheral blood. In addition autoreactive GAD65-specific T cells were identified in the initial pancreas transplant as well as in the peri-pancreatic transplant tissues (19). This patient received thymoglobulin, daclizumab, one dose of rituximab, plasmapheresis and intravenous immunoglobulin for therapy at the time of second pancreas transplant. Euglycemia persisted for three years during which time GAD65-specific autoreactive CD4 T cells could not be detected in the peripheral blood. However GAD65 autoantibodies and GAD65-specific autoreactive CD4 T cells were identified once again three years after the second pancreas transplant in the peripheral blood. After this point C-

peptide levels fell and were no longer detectable. Biopsy of the second pancreas transplant was performed that revealed a remarkably fibrotic pancreas transplant residual which had undergone rejection and fibrosis. We hypothesize that the autoimmune response may have been a factor in triggering rejection in this aggressively attacked fibrotic residual of pancreas transplant (19).

Potential Role of Memory T cells in T1DR

Of note, in the previously described patient, GAD65-specific autoreactive CD4 T cells had been detected with the same CDR3 and V-beta expression from the peripheral blood at the time of T1DR observation in the first transplant that persisted after loss of the second pancreas transplant. This is consistent with a memory T cell response that is resistant to conventional immunosuppression. This patient's course raises the concept that, in the event of re-transplantation for an SPK transplant recipient with T1DR, the previous pancreas transplant and peri-pancreatic transplant tissues which include lymph nodes, should be excised, since they may be harboring autoreactive, memory T cells. Removal of these autoreactive, memory T cell containing tissues, may provide protection from T1DR (19).

Another example of an SPK transplant recipient who developed T1DR provides further justification for following biomarkers including autoantibodies and autoreactive T cells, and specifically markers of T cell memory function. This patient presented with hyperglycemia nine years after SPK transplantation. Conversion for GAD65, IA-2, and ZnT8 autoantibodies occurred years prior to clinical hyperglycemia. Biopsy of the pancreas transplant demonstrated autoreactive T cells with insulitis and reduced insulin staining although some insulin staining of beta cells persisted. Infiltrating T cells predominantly expressed CD45RO, a marker of T cell memory. These T cells also stained positive for CD2. In the peripheral blood and in the peri-pancreatic transplant lymph nodes GAD65 autoreactive CD8 cells, the majority of which were memory T cells lacking surface expression of CCR7 (suggesting an effector memory phenotype) were identified. In view of evidence for T cell staining for CD2, we treated this patient with alefacept (a monoclonal antibody to CD2) (21). Alefacept has been used to treat patients with psoriasis and targets memory lymphocytes (22). Interestingly, alefacept has also been used in a clinical trial for patients recently diagnosed with T1D. In this study in which patients were given two three-month courses of alefacept, C-peptide secretion appeared to be preserved for up to two years (23,24). Our patient received one three-month course of alefacept during which time there was a remarkable fall in the frequency of circulating GAD65 and IGRP autoreactive memory CD8 T cells in the peripheral blood suggesting that alefacept had been effective from an immunologic perspective (25). However, alefacept was withdrawn from the market, and so we were unable to administer a second course of therapy which perhaps may have been successful. This patient experienced the return of memory autoreactive T cells, and has remained insulin-dependent.

Effects of Autoreactive T cells in Experimental In Vivo Model

Emphasizing the potential importance of autoreactive T cells as biomarkers, we studied the effect of CD4 autoreactive T cells that were specific for GAD65, that had been obtained

from two patients with T1DR, and placed them under the kidney capsule of immunodeficient (nude) mice along with human islet cells (19). Other mice received islet grafts alone or islet grafts plus irrelevant T cells from these two patients. The kidneys were removed one to two weeks later, and the grafts were examined. Control mice demonstrated normal islet cell histology and insulin staining. Grafts that received human islets plus GAD65 autoreactive CD4 T cells showed severe islet destruction and loss of insulin staining. In one instance where the mice were rendered diabetic after therapy with streptozotocin before transplant, those mice transplanted with human islets alone or islets with the irrelevant T cells became euglycemic. However mice that received islets along with GAD65 autoreactive CD4 T cells did not reverse their diabetes. This provided a functional correlate of the pathologic evaluation of these grafts. This study showed the potential for human GAD65 autoreactive CD4 T cells to mediate destruction of human islet cells in vivo (19).

Assessment of Autoantibodies in Miami PT Cohort

In our experience with SPK transplantation that began in 1990 and has extended for over 25 years, we have measured T1D-associated autoantibodies to GAD65, IA-2, and ZnT8 in serum samples that were collected longitudinally. Autoreactive T cells have been assessed in the peripheral blood and when possible in the pancreas transplant and in the peri-pancreatic transplant lymph nodes using tetramer technology (19,25). We recently completed a retrospective analysis of 223 SPK transplant recipients. In this study 17 patients (7.6%) were classified as having developed T1DR, 4.5% were categorized as having pancreas transplant chronic rejection and 9% as undetermined, since there was no apparent single cause for the hyperglycemia. Generally these latter patients had mild symptoms of diabetes, did not require insulin therapy, had evidence of persistent insulin secretion and were often overweight or obese lacking clear signs rejection. Importantly in our cohort T1DR appears to occur no less frequently than pancreas transplant chronic rejection, suggesting that monitoring of biomarkers of autoimmunity may serve a useful purpose.

Approximately 60% of our SPK recipients were classified as autoantibody negative at the time of transplantation, and 40% tested positive for autoantibodies (26). The presence of autoantibodies pre-transplant did not have an impact on outcome. Approximate 25% of our patients were classified as having autoantibody conversion i.e. they were autoantibody negative pre-transplant and became autoantibody positive on follow-up or they were positive for a specific autoantibody pre-transplant and later acquired positivity for other autoantibodies. Multiple autoantibodies are a risk factor for the development of T1D in relatives (27). Similar to the development of T1D in the native pancreas, autoantibodies are a risk factor for disease recurrence in the transplanted pancreas (26). Patients who developed T1DR had higher prevalence of all antibodies post PT (Figure 2). Importantly autoantibody positivity and multiple antibodies are associated with an increased risk of T1DR. The presence of three autoantibodies was associated with development of T1DR over 10 years in almost every instance. Furthermore, autoantibody conversion but not autoantibody persistence is a significant risk factor for T1DR (Figure 2). Of further note the appearance of autoantibodies to ZnT8 was followed by hyperglycemia within an average of six months.

Consistent with the genetic predisposition to islet autoimmunity and T1D, SPK recipients with T1DR carried the high risk HLA-DR 3/DR4 heterozygous genotype twice as often as SPK recipients that were normoglycemic. Similarly they shared HLA-DR alleles with their donors at an increased frequency. Finally carrying HLA-DR 3/DR4 genotype and sharing HLA-DR alleles with the donor were associated with autoantibody conversion (26).

Conclusion

In this review of biomarkers of PT both allo- and autoimmunity have been discussed. In our series of SPKT recipients who have developed T1DR, we have analyzed the cardinal features of autoimmune T1D, which have now been highlighted as the critical autoimmune biomarkers for PT. These include autoantibodies, autoreactive T cells, and PT biopsies (in which we have also demonstrated autoreactive T cells). Our effort has also been incorporated into the nPOD (network of Pancreas Organ Donors with Diabetes) program as nPOD-T (where T represents Transplantation) (28,29). We anticipate that further work focused on pancreatic-tissue-targeted research will lead to new therapeutic approaches to both T1DR and newly diagnosed T1D.

Acknowledgments

Financial support and sponsorship: Studies by the authors reviewed here were supported by grants from the National Institutes of Health (R01 DK070011, R01 DK052068), the JDRF (17-2011-594, 17-2012-3), the American Diabetes Association (RA-1-09-RA-413), the John C. Hench Foundation, and the Diabetes Research Institute Foundation, Hollywood, Florida.

Abbreviations

DKA	Diabetic Ketoacidosis
DSA	Donor Specific Antibody
ESRD	End Stage Renal Disease
GAD65	Glutamic Acid Decarboxylase 65
IA-2	Insulinoma Associated Protein 2
KT	Kidney Transplant
PT	Pancreas Transplant
SPK	Simultaneous Pancreas Kidney
T1D	Type 1 Diabetes
T1DR	Type 1 Diabetes Recurrence
T2D	Type 2 Diabetes
ZnT8	Zinc Transporter 8

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Key Bullet Point Sentences

1. Sudden onset of hyperglycemia (diabetic ketoacidosis) years after SPK (Simultaneous Pancreas-Kidney) transplantation, in the context of persistent urine amylase (bladder-drained pancreas transplant) and stable kidney transplant function, should alert to the possibility of recurrent auto-immunity.
2. T1DR (Type 1 Diabetes Recurrence) is most often preceded by a rise in levels of autoantibodies (GAD65, IA-2 and ZnT8), particularly levels of ZnT8 close to the event, and the greater the number of elevated autoantibodies the higher the likelihood of developing T1DR.
3. Genetic risk factors for T1DR include recipient HLA-DR3 and HLA-DR4, as well as donor-recipient sharing of HLA-DR3.
4. T1DR is characterized by the following: pancreas transplant biopsy that shows insulinitis, elevated serum autoantibodies, and auto-reactive T cells in the blood, pancreas transplant and peri-pancreas transplant tissues.
5. Despite numerous attempts to treat T1DR with various types of immunosuppression, similar to efforts to treat newly diagnosed T1D, none has yet proven to be effective.

Figure 1. Pancreas transplant biopsy in patient with T1DR. This shows an islet cell stained for insulin (brown), and CD3 (red). There is a CD3 infiltrate (insulitis) and persistent insulin staining, although there is evidence of Beta cell loss (Mag $\times$ 50).

Figure 2.

Kaplan-Meier analysis of T1DR-free survival according to autoantibodies on follow-up of SPK recipients. A Autoantibody positivity: positive vs negative. B Number of autoantibodies. C Autoantibody conversion. (Adapted from Reference 26: Risk factors for T1D recurrence in immunosuppressed recipients of simultaneous pancreas kidney transplants/Vendrame et al? American Journal of Transplantation/2016 Jan;16(1):235-45. doi: 10.1111/ajt.13426. Epub 2015 Aug 28.