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CASE REPORT

Vessel rejection secondary to human leucocyte antigen antibodies directed against the arterial conduit following pancreas transplantation from a separate donor

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Summary

Whole-organ pancreas transplantation is typically carried out using a Y-graft derived from the donor iliac vessels. We describe a case in which a 31-year-old male underwent a simultaneous pancreas-kidney transplant, but in which vessels from a different donor were used for the arterial anastomosis of the pancreas graft. Although initially there was good function, 18 months post-transplant the patient was admitted with diabetic ketoacidosis secondary to pancreas graft failure. Radiological investigations revealed complete occlusion of the vascular Y-graft, and laboratory investigations demonstrated donor-specific human leucocyte antigen (HLA) antibodies directed against HLA mismatches of the vessel donor. This case highlights the risks of using allogeneic vascular material for surgical anastomoses.

Introduction

Immunological graft failure remains problematic in allogeneic transplantation. The development of human leucocyte antigen (HLA) antibodies after transplantation, in particular those directed against donor HLA, has been associated with rejection and graft loss in many organ transplant studies [1–3]. Sensitisation to allogeneic HLA is commonly known to occur after pregnancy, blood transfusion or previous transplantation. However, allogeneic material is frequently used for vascular reconstruction in many surgical specialties, with little regard for its immunogenic potential.

Whole-organ pancreas transplantation is typically carried out using a Y-graft derived from the donor iliac vessels. We describe a case in which a 31-year-old male underwent a simultaneous pancreas-kidney (SPK) transplant, but in which vessels from a different donor were used for the arterial anastomosis of the pancreas graft. Although initially there was good function, 18 months post-transplant the patient was admitted with diabetic ketoacidosis secondary to pancreas graft failure. Radiological investigations revealed complete occlusion of the vascular Y-graft, and laboratory investigations demonstrated donor-specific HLA antibodies (DSA) directed against HLA mismatches of the vessel donor.

This case highlights the risks of using allogeneic vascular material for surgical anastomoses.

Case report

A 31-year-old male with type 1 diabetes of 20 years duration and end-stage renal failure requiring maintenance haemodialysis was assessed for simultaneous pancreas–kidney transplantation (SPK). The patient was blood group A+.

HLA typing was performed (Table 1), and HLA antibody status was assessed using Luminex technology (LABScreen Mixed, LABScreen PRA; One Lambda Inc., Canoga Park, CA, USA). The patient had not previously received a transplant or transfusion with blood products, and no HLA antibodies were detected in serum samples obtained 169 and 10 days prior to listing for a transplant. The patient was on the waiting list 699 days before transplant, and during this time, regular, 3 monthly serum samples were provided according to clinical protocol for HLA antibody detection. A total of eight samples were screened using Luminex technology (as above), and all were negative for HLA antibodies.

The patient received an offer of a blood group A+ kidney and pancreas from 24-year-old donor after brain death (DBD), HLA-A, B, DR mismatch grade 1, 1, 2, through the UK National Pancreas Allocation Scheme. The donor HLA type was confirmed locally (Table 1). At the time of transplant, the patient was unsensitized and the cross-match against the donor was negative by both complement-dependent cytotoxicity (CDC) and flow cytometry (FC). Evaluation of the organs before implantation revealed that no suitable vessels had been received to construct the arterial conduit. Concurrently, a second blood group A+ donor pancreas had been received for another patient. This organ was accompanied by sufficient length of donor vessels to enable both pancreases to be transplanted. The HLA type of the second donor was confirmed locally (Table 1), and the patient cross-matches against this donor were also negative by both CDC and FC. The absence of pretransplant DSA was subsequently confirmed using Luminex LABScreen Single Antigen Class I and Class II beads (One Lambda Inc.).

The operation was uneventful. Alumtuzumab induction and a standard protocol of maintenance immunotherapy comprising tacrolimus and mycophenolate were initiated. Postoperative haemorrhage required a further operation and transfusion of seven units of leuco-depleted packed red blood cells. The patient had good initial pancreas and kidney graft function and otherwise made an uneventful recovery. HLA antibody monitoring performed by Luminex technology 6 weeks later showed no evidence of *de novo* HLA antibodies. There were no other known sensitising events. The patient demonstrated good compliance, and there was no evidence of under-immunosuppression on regular drug level monitoring.

At 30 months post-transplant, the patient became acutely unwell with abdominal pain and vomiting and a diagnosis of diabetic ketoacidosis was made for which he was treated and then transferred to our centre. Radiological examination revealed complete occlusion sharply confined to the splenic and superior mesenteric arteries and no perfusion of the pancreas graft at all, which showed low attenuation and locules of gas, consistent with graft necrosis (Fig. 1). As the

Table 1. Recipient, simultaneous pancreas and kidney (SPK) donor and vessel donor human leucocyte antigen (HLA) types.

	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DRB3/4/5	HLA-DQB1*
Patient	02:01	07:02	07:02	01:01	DRB4*01:01	05:01
	03:01	51:01	15:02	04:02		03:02
SPK donor	01:01	07:02	07:02	15:01	DRB3*01:01	02:01
	03:01	08:01	07:01	03:01	DRB5*01:01	06:02
Vessel donor	02:01	***44:02***	05:01	***07:01***	DRB4*01:01	02:02
	29:02	***44:03***	16:01	15:01	DRB5*01:01	06:02

HLA targets of donor-specific antibodies detected at 30 months highlighted in bold italic type.

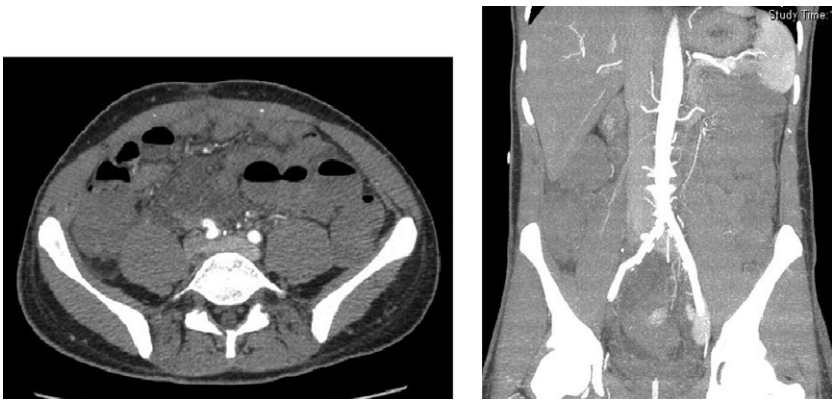

Figure 1 Computed tomography images of occluded Y-graft and nonperfused pancreas graft.

patient was pain-free and stable, with no remaining salvageable pancreas graft function, neither a biopsy nor pancreatectomy was indicated, and he was discharged on insulin. His kidney graft continued to function, and he remained dialysis-independent.

Human leucocyte antigen antibody testing on admission using Luminex LABScreen Single Antigen Class I and Class II beads (One Lambda Inc.) revealed HLA class I antibodies with median fluorescence intensity (MFI) values >7000 reactive with HLA-A29, A43, B44, B45 and B82. HLA-A29 and B44 are mismatched antigens from the vessel donor. The antibody specificity analysis and MFI values are shown in Fig. 2. HLA class II antibody testing revealed weak reactivity against HLA-DR7 (MFI 1026), which was one of the mismatches from the vessel donor.

Discussion

In pancreas transplantation, the graft is usually implanted using a Y-graft formed from donor iliac arteries to anastomose the donor superior mesenteric artery (SMA) and splenic artery (SA) to recipient common iliac artery. This allows size-matching of recipient SMA and SA to donor iliac vessels of a similar calibre [4] and reduces prereperfusion operative time during which the transplant graft may accrue damage. Donor vessels are also frequently used for complex arterial anastomoses in other surgical areas [5]. Allogeneic material is widely used in surgery as decellularised biological grafts developed as meshes for hernia repair [6] and as vessels for dialysis access [7]. Indeed human allogeneic material may be preferred over synthetic graft materials, which carry an infection risk [8].

Donor-specific antibodies (DSA) frequently develop after kidney transplantation and confer poorer graft survival [9], particularly when associated with acute rejection [10]. Immunological graft loss remains a challenge in pancreas transplantation [11], and the role of *de novo* DSA after pancreas transplantation has not been clearly established. Circulating DSA can initiate antibody-mediated rejection (AMR), which is diagnosed by their presence alongside morphological evidence of microvascular tissue injury, often, but not universally, associated with C4d staining. Endothelial cells are involved in initiating the allo-immune response and damage to the endothelium is a prominent histological feature of AMR, typically focused within the endothelial capillary network [12]. Studies examining donor tissue have shown endothelial cells to express both HLA class I and class II molecules, as well as the costimula-

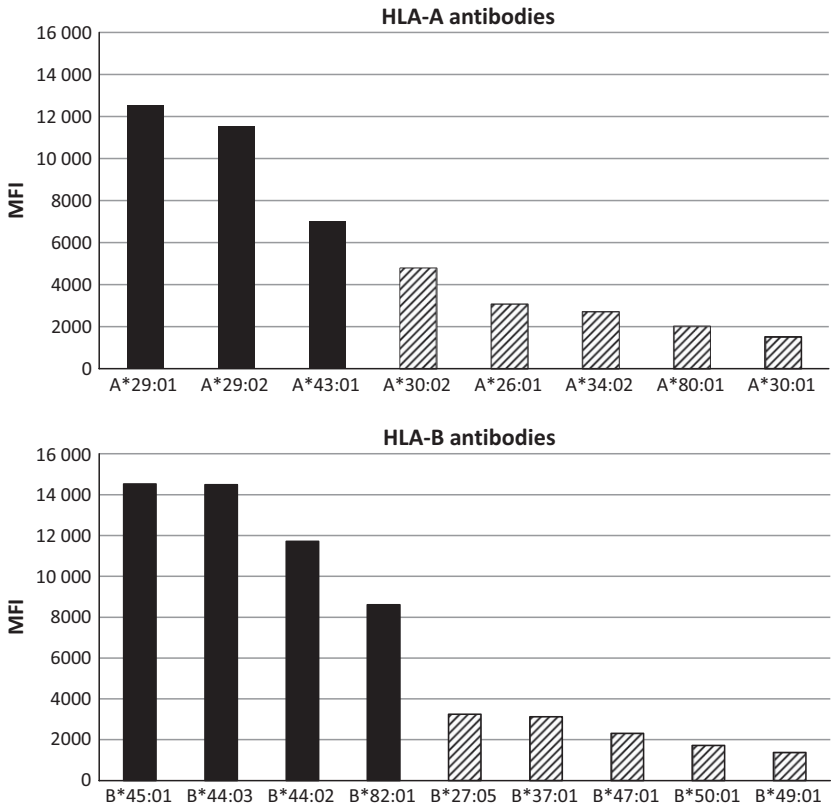

Figure 2 Human leucocyte antigen (HLA) antibody specificity analysis performed 30 months post-transplant using Luminex single antigen beads. Median fluorescence intensity (MFI) values of binding to HLA-A and HLA-B antigen-coated beads.

tory molecule LFA-3, endowing these cells with the capacity to present antigen to recipient T cells [13]. The endothelium is therefore important in the allo-immune response both as stimulator target [14].

Arterial lesions are commonly seen in transplanted organs with chronic rejection, as described in the heart as transplant arteriopathy, the lung as bronchiolitis obliterans syndrome, the liver as vanishing bile duct syndrome and in the kidney as arteritis. Much of the current research in human subjects has focussed on investigating chronic changes in microvascular endothelium, while the role of macrovascular endothelium in initiating immune responses has not been clearly described. While the role of endothelium in the immune processes involved in AMR has been demonstrated, there are anatomical and phenotypical differences between microvascular and macrovascular endothelium. A biopsy study in 33 heart transplant recipients demonstrated that microvascular and macrovascular pathology did not develop in parallel [15], and acute rejection of vascular allografts has not been clearly demonstrated.

Immunological changes associated with vascular allografts have been observed. Venous allografts have been shown to initiate immune responses in dogs [16]. Histological analysis of excised saphenous vein allografts showed cellular infiltration in patients both with and without low-dose immunosuppression. Patients developed *de novo* and increased titre anti-HLA antibodies although specificities were not determined and sensitising events such as transfusions were not described [17]. Lopez-Capero *et al.* observed that patients receiving deceased donor arterial and venous allografts used for haemodialysis access developed HLA class I and class II antibodies. However, HLA typing of the allograft donor was not performed, and the timing of antibody development and other sensitising events were not described, thus the development of antibodies could not be directly attributed to the graft [18]. In the case we report, although histology was unavailable as resection or biopsy of the conduit was not performed, development of post-transplant antibody directed against the vascular allograft and not the pancreas graft has been demonstrated through pre- and post-transplant antibody profiling. We have described the clinical course of the patient, including the absence of sensitising events or periods of under-immunosuppression. Despite significant immunosuppression, previously undescribed, complete occlusion of the Y-graft occurred depriving the pancreas graft of its vascular supply. As the use of a Y-graft from a different donor is not common practice in our centre, it is not known whether the use of two donors contributed to the rejection in this case.

In this case report, we describe a case of arterial thrombosis resulting in graft failure, which was associated with (and probably secondary to) the development of DSA. To our knowledge, this is the first case directly comparing vascular allograft donor HLA typing and recipient HLA antibody specificities to identify the presence of *de novo* DSA against a macrovascular allograft target resulting in organ transplant rejection.

Authorship

All authors contributed to the analysis of data and drafting and revision of the manuscript.

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