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CASE REPORT

Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation

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Summary

We describe the presumably first intentional ABO-incompatible deceased-donor kidney and pancreas transplantation with a severe antibody-mediated rejection during a rebound of isoagglutinins. Rejection was successfully treated with eculizumab, which inhibits the terminal pathway of complement. Complement analysis (C3, C3d,g, and a modified assay of classical complement-related hemolytic function) documented complement activation and confirmed that eculizumab completely blocked complement function. At 6 months, the patient had normal kidney and pancreas function, and histological evaluations revealed no evidence of sustained graft damage. This successful transplantation suggests that ABO barriers can safely be overcome without extensive preconditioning, when the complement inhibitor eculizumab is included.

Introduction

Pancreas transplantation provides a cure for patients with severe diabetes, with excellent long-term results [1]. However, in our experience, efficient local allocation of pancreas allografts is sometimes hampered by the need for blood-type compatibility between donor and recipient. In this respect, pancreas allografts have been discarded in some cases because of a positive donor-specific crossmatch in blood group-compatible recipients. On the other hand, crossmatch-negative recipients with an incompatible blood group have not been considered suitable candidates. We have now assessed transplantation across ABO barriers as a possible strategy for addressing the apparent underutilization of potentially suitable pancreas allografts. The current case delineates a strategy for prompt reversal and monitoring of complement activation in the event of humoral immune response after ABO-incompatible transplantation.

Patient and methods

A 43-year-old Caucasian man suffering from poorly controlled Type I diabetes mellitus and terminal renal failure

underwent an ABO-incompatible (phenotype A1 to O) PNTx with grafts from a deceased 29-year-old donor. The baseline isoagglutinin titers were 1:4. The complement-dependent cytotoxic- as well as flow-cytometric cross-matches were negative. Serial Luminex assays of serum samples from days 0, 10, 38 and 53 were performed and excluded the presence of donor-specific HLA antibodies.

Antibody removal and immunosuppression

A single session of plasma exchange was performed prior to the transplantation followed by serial antigen-specific immunoadsorptions (IA) (Glycorex Transplantation AB, Lund, Sweden). Isoagglutinin titers were assessed using the gel card method [2]. Rituximab at 375 mg/m^2 body surface area was given on days 0. As a result of an incomplete depletion of CD20-positive B-cells (measured using flow-cytometry immunophenotyping), a second dose of rituximab was given on day 7 resulting in total depletion of remaining B-cells. Basiliximab at 20 mg was given as induction treatment on days 0 and 4. Immunosuppression included tacrolimus (12-h target trough level 10–12 ng/ml), mycophenolate mofetil [12-h target 'area under the curve' 30–50 (mg/l) × h] and steroids. Eculizumab was used as rescue treatment for antibody-mediated rejection (AMR) at 600 mg/day on days 10 and 14.

Complement analysis

C3d,g was analyzed using enzyme-linked immunosorbent assays (ELISA) [3]. C3 was assessed using nephelometry (Beckman Coulter Immage, Ramsey, MA, USA). The C3d,g/C3 ratio was calculated as an indicator of complement activation [4,5]. A modified single-tube assay to detect classical complement pathway (CPP)-related hemolytic function was performed as previously described [6].

Results

The initial postoperative recovery was uneventful. IA-treatments were performed on days 0 and 1, and thereafter isoagglutinins were zero. On day 6, isoagglutinin levels were elevated, and IA was started on a daily basis (Fig. 1a–e). Despite intensified IA-treatments, isoagglutinin titers increased to 1:32 on day 9. Concomitantly, the patient presented with acute abdominal pain accompanied by rapid increase of C-reactive protein. A re-operation excluded graft-related surgical complications. An intra-operative kidney biopsy showed peritubular capillaritis with dilated vessels containing inflammatory cells, a moderate amount of macrophages in the interstitium and a distinctive peritubular C4d deposition (Fig. 2a–c). Luminex assays showed no donor-specific HLA antibodies. On day 10, the patient received 600 mg eculizumab, and the CPP value dropped to 0% on day 11. The second and final dose of eculizumab (600 mg) was given on day 14, when CPP value increased to 20%. On day 18, CPP values consistently increased to normal range. IA was discontinued on day 25.

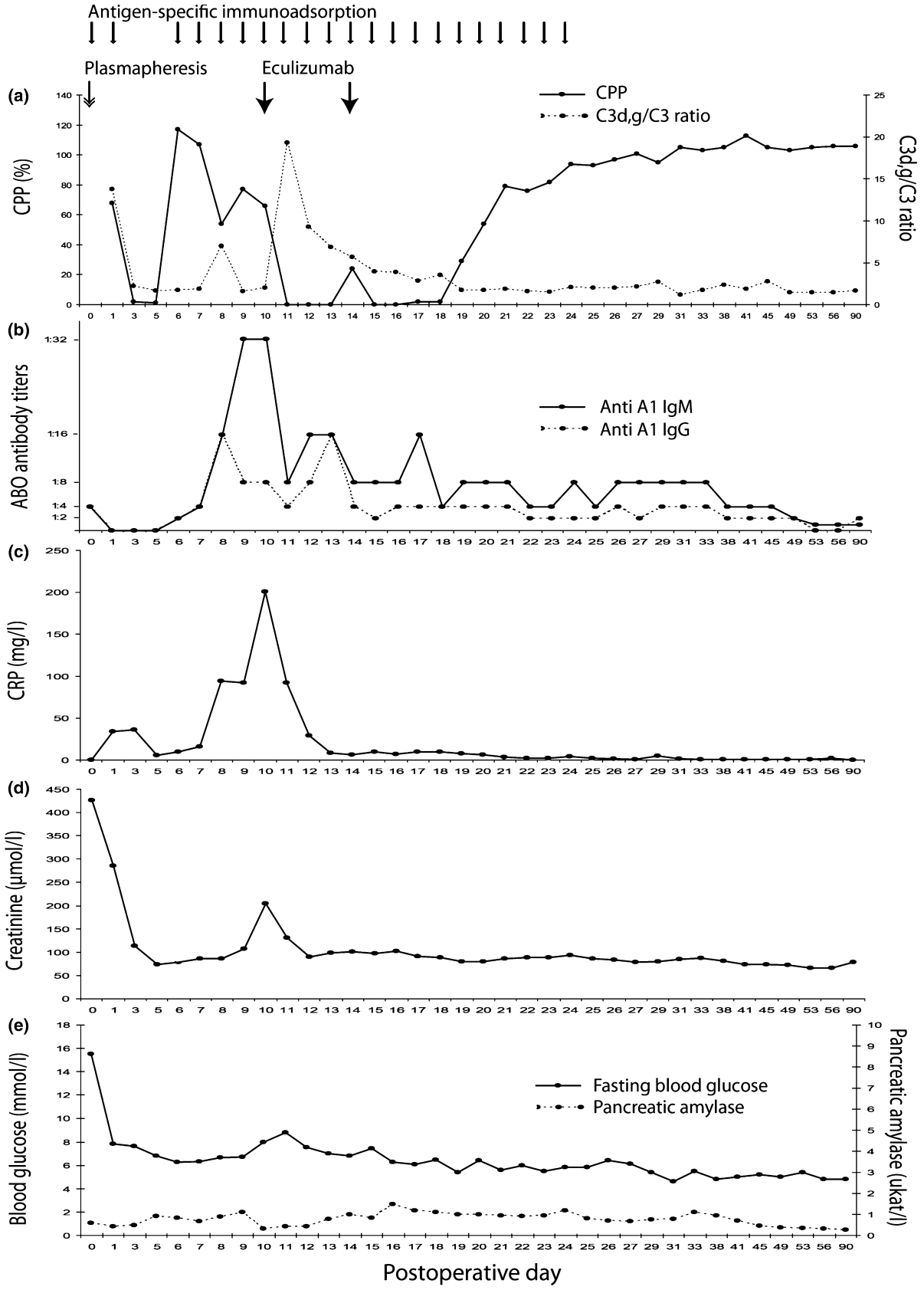
At 3 months, biopsies of the kidney and the attached duodenal segment of the pancreas showed no signs of rejection. At the 6-month follow-up, the patient had a plasma creatinine of 77 μmol/l, cystatin-C GFR of 106 ml/min/1.73 m^2, HbA1c of 37 mmol/mol and normal oral glucose tolerance. A further biopsy of the attached duodenal segment at 6 months showed normal histological architecture and no inflammation.

As a result of the increased risk for *Neisseria meningitidis* in connection with eculizumab treatment [7], the patient received 3 months of prophylactic treatment with phenoxymethylpenicillin at 1 g/day.

Discussion

The role of antibodies in AMR involves the activation of the complement cascade via the classical pathway cleavage of C3 into C3b and C3a, and the downstream formation of the membrane attack complex SC5b-9 [8]. This mechanism has been shown to be responsible for the speed and severity of AMR and cell injury [9–11]. Eculizumab is a humanized monoclonal antibody against complement factor C5 that prevents generation of SC5b-9 during complement activation [12,13].

Figure 1 (a–e) Characterization of complement activation and response to eculizumab during antibody-mediated rejection after an ABO-incompatible pancreas and kidney transplantation. Complement activation was characterized by means of the C3d,g/C3 ratio and a modified assay of the hemolytic function of the classical complement pathway (CPP) (Panel a). Five days after the transplantation, isoagglutinin titers consistently increased (Panel b) despite intensive antigen-specific immunoadsorption treatments. On day 10, the patient presented with a rapid increase of both C-reactive protein (CRP) (Panel c) and creatinine (Panel d). Repeated Luminex assays showed no donor-specific HLA antibodies. Kidney biopsy revealed antibody-mediated rejection, and the patient received the first dose of eculizumab. Subsequently, kidney function and CRP were normalized, and the CPP assay indicated total suppression of complement function. Short-term suppression of complement activity was considered important to promote accommodation. Hence, the final and pre-emptive dose of eculizumab (600 mg) was given when the CPP value indicated recovery of complement function on day 14. Starting on day 18, the CPP value returned to normal range, indicating fully recovered complement function. However, no further complement activation or deterioration of kidney function was observed. The pancreas graft was unaffected during the rejection event and the remaining follow-up period (Panel e).



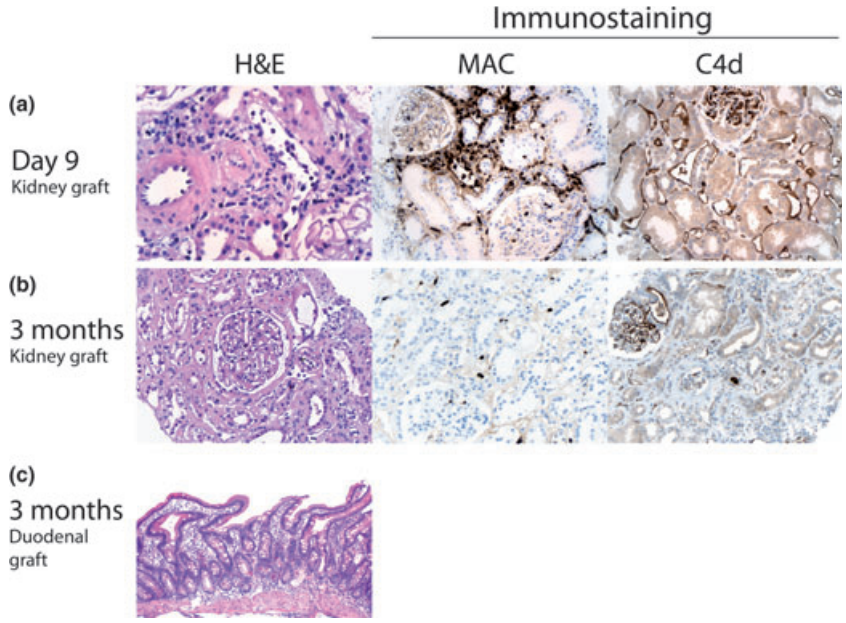


Figure 2 (a–c) Histological evaluation of biopsies from the kidney and duodenal segment of the pancreas. Panel a: Kidney biopsy on day 9 showed peritubular capillaritis with dilated vessels containing inflammatory cells [left: hematoxylin and eosin (H&E) staining], a moderate amount of macrophages in the interstitium [center: immunohistochemical staining for myeloid-histiocyte antigen (MAC) expression] and a strong general C4d staining of the peritubular capillaries (right). Panel b: Kidney biopsy at the 3-month follow-up revealed normal histology (left) with scattered single macrophages (center) and moderate C4d positivity in a minority of capillaries (right). Panel c: Biopsy from the duodenal segment graft at the 3-month follow-up showed normal villous architecture and no increased inflammation. A further duodenal biopsy at 6 months confirmed normal histology and the absence of sustained graft injury (picture not shown).

In this report, we describe an intentional ABO-incompatible deceased-donor PNTx between a blood Type A1 donor and 0-recipient. As a result of its documented significance during the humoral immune response, we monitored complement activation by determining the C3d,g/C3 ratio and CPP percentage, and we included eculizumab as rescue treatment to immediately block complement function at the C5 level in the event of AMR.

In deceased-donor transplantation logistics during organ allocation and the legitimate demand for short ischemia time restrain the possibilities for time-consuming preconditioning. In our experience, the preoperative management can therefore not include more than one plasma exchange pre-transplant. In the current report, the recipient was selected because of his low baseline isoagglutinin titers, and therefore there was no mandatory need for a preoperative plasma exchange. However, we considered the following aspects of the perioperative management to be important to increase the safety margin of the procedure: (i) induction with single plasma exchange and rituximab and (ii) maintenance immunosuppression adopted from a successful protocol for ABO-incompatible living donor kidney transplantation (LDKT) including regular postoperative antigen-specific IA [14]. Given this strategy, we assumed AMR to be unlikely, and therefore chose to use eculizumab primarily as a rescue treatment.

Despite the measures taken to minimize the risk of humoral complications, the patient experienced a distinct biopsy-verified AMR on day 9, when the isoagglutinins increased to 1:32. The occurrence of humoral rejection during rebound of isoagglutinins and the absence of HLA-antibodies prompted us to assume ABO reactivity. However, a non-HLA mediated humoral rejection such as a vascular endothelial-cell antigen–antibody mediated rejection could not be excluded.

The pivotal role of complement as the key inflammatory mediator during AMR was demonstrated by the observation that both the kidney function and the severe inflammatory response were rapidly normalized after a single administration of eculizumab (600 mg) on day 10. The rapid drop in the CPP value to 0% and the consistent decrease in the C3d,g/C3 ratio also pointed to a distinct complement-inhibitory and anti-inflammatory effect of eculizumab (Fig. 1a–e).

Accommodation is a well-known phenomenon that is defined as the absence of graft injury despite the presence

of antigen on endothelial cells and the corresponding antibody in the circulation. In ABO-incompatible LDKT, short-term protection from AMR is provided by temporary removal of isoagglutinins during preconditioning. In this scenario, accommodation is established within 1–2 weeks after transplantation [15]. However, unlike LDKT, whole pancreas transplantation has similarity with composite allograft transplantation, as it consists of pancreatic and intestinal tissue. According to a United Network for Organ Sharing (UNOS) registry survey, four ABO-incompatible intestine transplantations have been performed until 2009 and all of them have failed [16]. Kenmochi *et al.* presented more encouraging outcome after living-donor segmental pancreas transplantation in three patients undergoing preconditioning [17]. On the basis of these limited data, we considered the attached duodenal segment of the pancreas to be more vulnerable to graft injury during ABO reactivity. Considering the empirical knowledge of accommodation, we reasoned that short-term protection from AMR by suppression of complement function might be beneficial to reduce the risk for sustained graft injury in the kidney and the composite tissue of the pancreas allograft. Hence, we used CPP as an analytical tool to monitor complement function and to guide the second pre-emptive administration of eculizumab, which was given on day 14, when the test indicated recovery of the complement function. After 1 week of total suppression, the patient's complement function had recovered and reached full capacity within a few days.

At the 3-month and 6-month follow-up, the patient had normal pancreatic and renal function. Furthermore, the histological evaluations of the kidney and the attached duodenal segment of the pancreas showed no rejection and confirmed the absence of sustained graft injury.

This report exemplifies the pivotal role of complement as the key inflammatory mediator during AMR. It indicates that ABO-incompatible deceased-donor transplantations, *per se*, are likely to be feasible and safe, provided that the protocol includes eculizumab as complement activation inhibitor. Furthermore, this is the first report suggesting that pancreas and its attached duodenal segment might not be vulnerable to sustained graft injury after ABO-incompatible transplantation with a severe rejection.

Authorship

ARB: designed and performed the study, collected and analyzed data and wrote the paper. TN, BZ, MW, CB, AW and AM: performed the study. BN and GT: performed the study, contributed to the design and analyzed the data.

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