

Delayed hyperacute rejection in a patient who developed *Clostridium difficile* infection after ABO-incompatible kidney transplantation

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Abstract: Over the past decade ABO incompatible transplantation has emerged as an important potential source for increasing living kidney transplantation in selected transplant centers. Early reports suggest that patients who have elevated serum anti-blood group antibody titers (anti-A/B) before transplantation and a rebound antibody production after antibody removal may be at high immunological risk. With currently available immune modulation protocols and immunosuppressive therapy, excellent short- and long-term patient and graft survival rates have been achieved even in those with high anti-A/B antibody titers before plasmapheresis or immunoadsorption. Nonetheless, acute infection with an organism possessing surface markers analogous to blood group antigens such as carbohydrate structures on the surface of bacterial cell wall occurring before the firm establishment of accommodation can trigger the onset of acute antibody-mediated rejection. We herein report a case of delayed hyperacute rejection in an A1 to O, ABO incompatible transplant recipient following an episode of *Clostridium difficile* infection.

Keywords: ABO incompatible transplantation, delayed hyperacute rejection, kidney transplantation, *Clostridium difficile* infection

In ABO incompatible transplantation, acute antibody-mediated rejection can be classified into hyperacute (<24 hours), delayed hyperacute (<1–7 days), and acute (<3 months). Hyperacute rejection generally does not occur if the recipient receives appropriate pretransplant desensitization and immune modulation therapy to lower anti-blood group antibodies before transplantation and immunosuppressive therapy following transplantation to suppress further antibody production. However, delayed hyperacute rejection may occur up to one week posttransplant during the recovery phase of injured vascular endothelial cells within the graft following reperfusion. During this period, the active production of blood group glycosyltransferase by vascular endothelial cells, the increased antigenicity of vascular endothelial cell surface antigens, and the increased production of antibodies by the recipient can trigger delayed hyperacute rejection. Antibody removal procedure prior to the development of delayed hyperacute rejection and suppression of antibody production during this critical period with currently available immunosuppressive therapy can prevent acute antibody-mediated rejection (AMR) and allow accommodation to establish in most cases. Nonetheless, postoperative infections, particularly sepsis, can trigger the onset of acute antibody-mediated rejection.¹ We herein report a case of delayed hyperacute rejection in a patient who developed *Clostridium difficile* (*C. difficile*) infection in the first week after transplantation and discuss potential causes of delayed hyperacute rejection.

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Case report

A 39-year-old man, blood type O, who had type 1 diabetes mellitus since the age of 1 and end-stage-renal disease secondary to biopsy-documented interstitial nephritis underwent a living-related renal transplant from his father in 1990 at the age of 21. The transplanted kidney was placed in the right iliac fossa. In 1995, he had two episodes of acute cellular rejection which led to eventual allograft failure and return to dialysis a year later. His immunosuppressive therapy was gradually tapered and discontinued. However, he subsequently developed severe graft pain with associated fever and malaise and required allograft nephrectomy. Pathology demonstrated acute cellular and humoral rejection and chronic transplant rejection with chronic transplant glomerulopathy. In 1997, he underwent simultaneous pancreas and kidney transplantation. The kidney allograft was placed in the left iliac fossa, with the enteric drained pancreas placed on the right. He had an uncomplicated postoperative course and good kidney and pancreas allograft function. His immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil, and prednisone. His kidney allograft functioned well until May 2008 when he developed acute cellular rejection that led to graft loss despite antithymocyte therapy. His pancreas allograft has continued to function well and he has remained insulin independent. In July 2009, he underwent sequential allograft nephrectomy and A1 to O, ABO incompatible living related re-allograft transplant from his maternal cousin after a successful desensitization and immune modulation protocol. Preimmunomodulation anti-A1 titer was 1:256 for IgG and 1:32 for IgM. He is 2HLA-A, -2DR mismatched with his cousin. Although the patient displayed historical weak donor specific antibody (DSA) to DQ5 (MFI = 2272) and DQ6 (MFI = 3632), single antigen bead Luminex assay before transplantation showed no evidence of either class I or class II donor-specific anti HLA antibodies. Furthermore, the pretransplant pronase T- and B-cell flow cytometry crossmatch was negative. CD19 and CD 20 were <1% at the time of transplant. He was given Thymoglobulin induction and had immediate graft function with greater than 5 liters of urine output the first postoperative day. His medications included tacrolimus, solumedrol 250 mg tapering, dopamine at 3 g per kg per mm² per hour, furosemide at 10 mg per hour, ganciclovir 100 mg IV daily adjusted for renal function, trimethoprim/sulfamethoxazole 80/160 mg p.o. daily, fluconazole 400 mg daily, and cefazolin 1 g intravenous every 8 hours × 24 hours. His exam on postoperative day 1 (POD#1) was remarkable for incisional pain and absence of bowel sounds. His vital signs were stable. Daily anti-A1

immune titers were obtained and plasmapheresis performed when anti-A1 titer was $\geq 1:16$ per our center protocol. On POD#5, the patient developed diarrhea and fever to 38.3°C for which he was started on metronidazole 500 mg po tid empirically. No change in immunosuppressive therapy was made. *C. difficile* toxins A and B were detected in the patient's stool specimen by enzyme-linked immunoassay. Treatment with metronidazole resulted in resolution of diarrhea and fever and negative repeat toxin assays 4 weeks following treatment.

The patient had good initial graft function with creatinine decreasing to 1.7 mg/dL on POD#6. Nonetheless, plasmapheresis was performed on POD#6 for an anti-A1 IgG titer of 1:32 per our protocol. On POD#7, however, his serum creatinine rose to 1.9 mg/dL. Anti-A1 IgM titer increased from 1:8 to 1:32 whereas anti-A1 IgG remained unchanged. Over the next 24 hours (POD#8), his creatinine increased further to 4.4 mg/dL concomitant with a sharp rise in both IgM and IgG titers despite plasmapheresis the preceding day (IgM 1:32 to 1:128 and Ig G 1:32 to 1:256). Platelet count decreased to 63 (from a preoperative level of 154) while LDH was within normal range. Clinically, the patient became oliguric with a sudden drop in urine output to between 30 mL and 40 mL an hour. His urine was dark red and he developed mild to moderate graft tenderness. A stat Doppler ultrasound revealed little to no flow within the kidney cortex prompting exploratory laparotomy. Intraoperatively, the kidney was found to be enlarged, measuring 16 cm × 9 cm, with flow in the renal artery and the renal vein and no evidence of thrombosis on the main vasculature. However, the kidney appeared dark, enlarged, and firm. A tentative diagnosis of hyperacute rejection was made and allograft nephrectomy was performed. Pathology demonstrated severe antibody mediated rejection, consistent with delayed hyperacute rejection (Figure 1). Anti-HLA antibodies testing performed on day 1 after the allograft nephrectomy showed weak donor specific antibodies to DQ5 (MFI 1518), DQ6 (MFI 1936), and de novo DSA to HLA-A1 (MFI 5151). No sera were tested for Anti-HLA antibodies prior to transplant nephrectomy. A summary of the patient's clinical course is shown in Figure 2.

Discussion

Since 2008, our center has performed ABO incompatible transplant using a preconditioning regimen consisting of rituximab, plasmapheresis (PP), and intravenous immunoglobulin (IVIG), and tacrolimus and mycophenolate mofetil maintenance immunosuppression. Our initial experience has included successful transplantation from blood groups

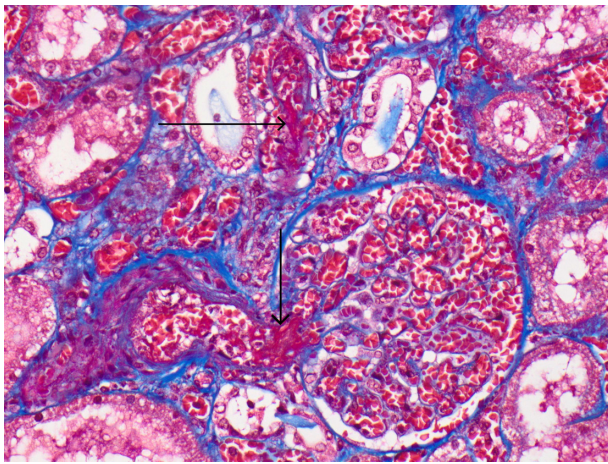


Figure 1 Arrows demonstrate fibrin thrombi in glomerular hilum and adjacent arteriole (Masson trichrome, original magnification 400x).

A1, A2, AB, and group B living donor kidneys. To date, 17 patients have undergone ABO blood type incompatible kidney transplantation with preimmunomodulation IgG titers as high as 1:1024. Our recipients have been primarily blood group O (71%). Cumulatively, we have had one episode of cellular rejection and no cases of humoral rejection. Serum creatinine at discharge was 1.3 ± 0.5 mg/dL, at 1 month 1.4 ± 0.4 mg/dL, at 3 months 1.2 ± 0.3 mg/dL, at 6 months 1.1 ± 0.3 mg/dL, and at 1 year 1.2 ± 0.2 mg/dL. Our patient survival rate is 100% with a graft survival rate of 94% (unpublished results). In the present case report, the patient had immediate graft function with excellent urine output and appropriate decrease in creatinine to 1.7 mg/dL on POD#6. Plasmapheresis was performed on POD#6 and POD#7 for an anti-A1 IgG titer of 1:32 per our center protocol. Nonetheless, on POD#8, there was a sharp rise in both IgM and IgG anti A1 titers (IgM 1:32 \rightarrow 1:128 and IgG 1:32 \rightarrow 1:256), associated with a rise in serum creatinine, acute oliguria, a drop in platelet count, and graft tenderness, all of which were suggestive of the onset of acute rejection. Allograft nephrectomy confirmed delayed hyperacute rejection.

Takahashi was the first to propose that the changes in anti-A or anti-B antibody titers after the onset of acute AMR rejection differ between patients whose grafts were lost and those whose grafts survived.² In patients with graft loss, an increase in IgG antibody titers is usually accompanied by a parallel increase in IgM antibody titers. In contrast, in those who respond to treatment without functional graft loss, IgM titers are usually increased without an appreciable change in IgG antibody titers. These findings suggest that there is a difference between primary and secondary immunological responses, ie, between primary

sensitization and resensitization. The latter is seemingly caused by ABO-blood group antigens, while the former is caused by ABO-blood group-associated antigens.² ABO-blood group-associated antigens have been found to exist on the surface of certain bacterial cells and food products. It has been suggested that, after entering the body, these antigens become cross-reacting antigens, causing sensitization and antibody production leading to acute antibody-mediated rejection (AMR).¹⁻² *Klebsiella pneumoniae* (*K. pneumoniae*) urosepsis after B to O incompatible transplant leading to acute AMR has been described.¹ It is speculated that ABO-antigen-like substances on the surface of *K. pneumoniae* cross-react with the recipients's antigens, causing primary sensitization and anti-B antibody production. These antibodies react with vascular endothelial-cell B antigens, thereby triggering acute antibody-mediated rejection. In the present report, the patient developed *C. difficile* diarrhea and low-grade temperature on POD#5. On POD#6 there was a 2-fold rise in both antiA1 IgG and IgM titers (IgG: 1:16 to 1:32 and IgM 1:4 to 1:8). Nonetheless, his allograft function continued to improve with serum creatinine decreasing to 1.7 from 2.0 the day prior. On POD#7, his serum creatinine rose to 1.9 mg/dL and anti A1 IgM titer increased from 1:8 to 1:32 despite his having received plasmapheresis the preceding day, whereas anti A1 IgG titer remained unchanged. The temporal relationship between *C. difficile* infection and a subsequent 2-fold rise in IgM titer (and unchanged IgG titer) despite plasmapheresis suggests that de novo antibodies to *C. difficile* (IgM) may have triggered delayed hyperacute rejection. Alternatively, an initial parallel rise in antiA1 IgG and IgM titers on POD#6 suggests that delayed hyperacute rejection may have been triggered by resensitization (IgG) coupled with de novo antibodies to *C. difficile* (IgM). Although no sera were tested for anti-HLA antibodies prior to transplant nephrectomy, the contributory role of DSA in the development of hyperacute rejection cannot be entirely excluded. Nonetheless, their very low levels in the postnephrectomy serum made the latter possibility unlikely.

It has been suggested that acute AMR due to primary sensitization usually progresses more slowly and is less severe than that triggered by resensitization caused by ABO-blood-group antigens. Nonetheless, in the present case, it is conceivable that the occurrence of *C. difficile* infection during the critical period (ie, before accommodation can be established) may have triggered the development of a delayed hyperacute rejection. In ABO incompatible transplantation, accommodation can be loosely defined as survival of the graft without acute AMR despite the presence of blood group antigens on graft endothelial

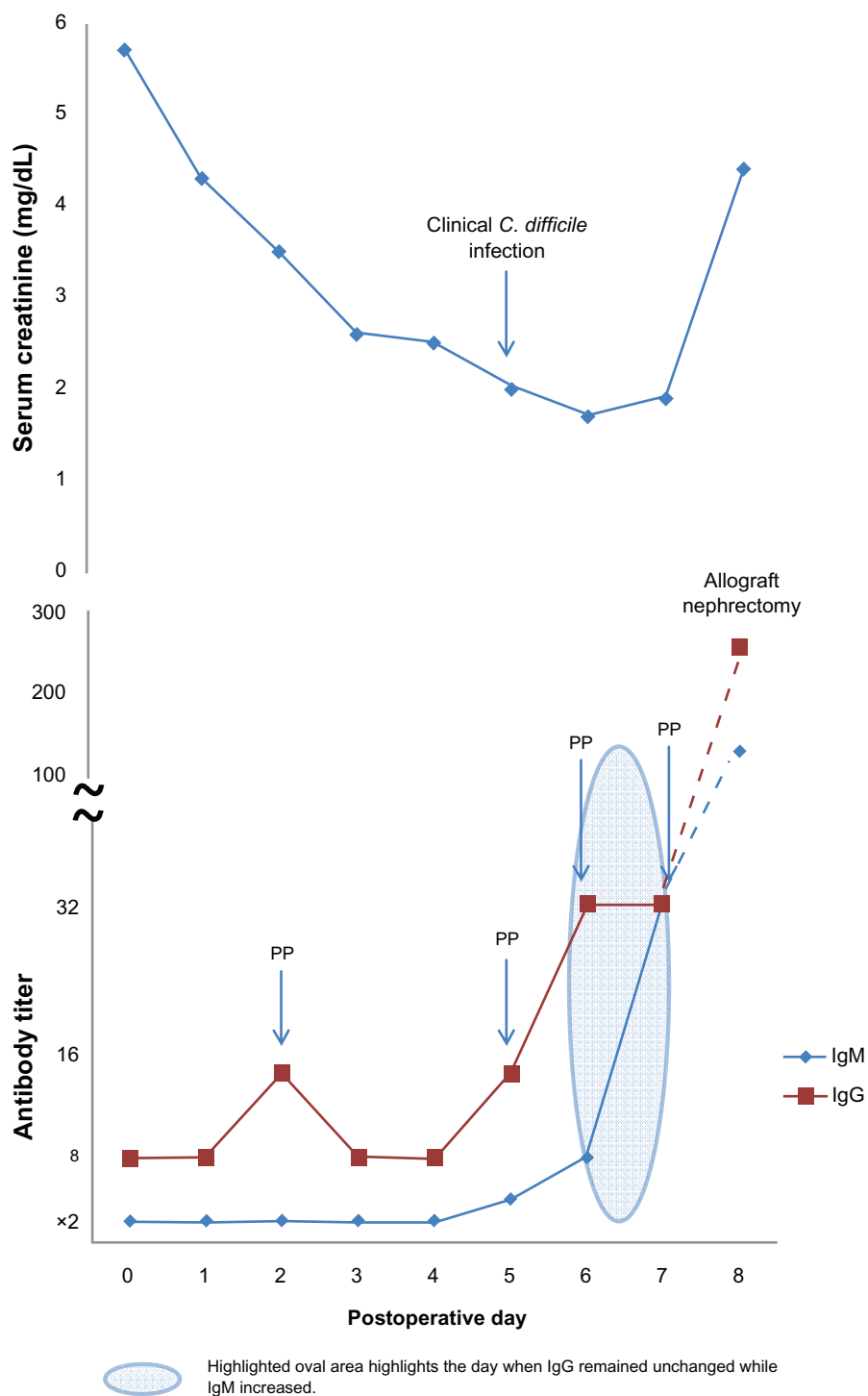
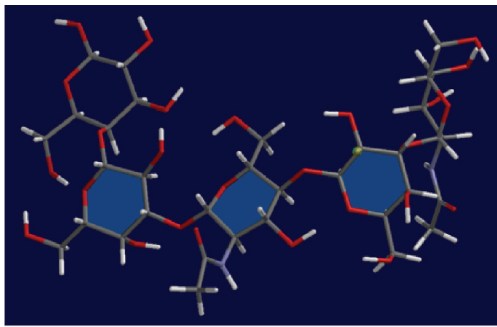


Figure 2 Patient's clinical course.
Abbreviation: PP: plasmapheresis.

cells and the presence of their corresponding antibodies in the recipient's blood.³ Accommodation is generally not induced or established until 1–2 weeks after transplantation. It should be noted that the risk of acute antibody-mediated rejection due to primary ABO-blood-group-associated antigens cannot be predicted on the basis of low titers of serum anti-ABO antibodies before transplantation.

Natural antibodies against bacterial polysaccharide or carbohydrates are thought to arise by a T-cell-independent mechanism owing to the aggregation of B-cell receptors in response to exposure to the repetitive structure of polysaccharide antigens.⁴⁻⁵ In our case, we speculate that the recipient became sensitized to the carbohydrate antigens of *C. difficile*, which resemble the ABO blood type antigens,



Antigen A

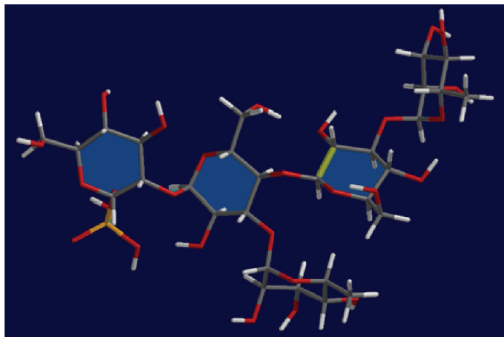
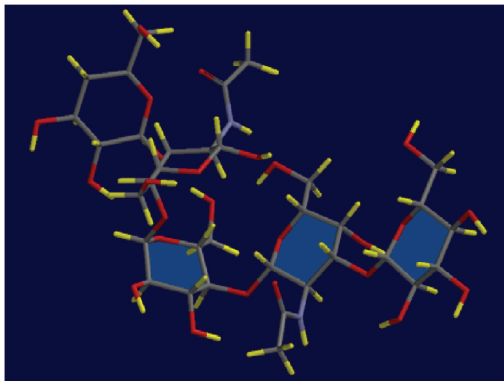
*C. difficile* PSI*C. difficile* PSII (end carbohydrate mannose removed for better view)

Figure 3 Conformers for Antigen A and two types of *C. difficile* are shown. Calculations were carried out at the Molecular Mechanics MMFF levels using Spartan 06 software. Three carbohydrates that are structurally similar are highlighted in blue.

resulting in cross-reaction (Figure 3). This led to a rapid increase in anti-A antibody production that reacted with type A antigens on the surface of the graft vascular endothelial cells, which elicited acute AMR.

It is also tempting to speculate that *C. difficile* infection may promote a generalized immune response in a fashion similar to that seen with acute rejection. Recently, C-terminal repeating sequences of *C. difficile* toxin A has been shown to induce the production of chemokine and adhesion molecules in endothelial cells and promote migration of leukocytes.⁶

The causal relationship between *C. difficile* infection and delayed hyperacute rejection remains speculative. Nonetheless, it should be noted that antibiotic-associated *C. difficile* (particularly cephalosporins, ciprofloxacin, and amoxicillin-clavulanate) has become a serious epidemiologic problem worldwide. In contrast, it has been suggested that the development of bacterial infection, particularly sepsis, occurring before the firm establishment of accommodation can potentially trigger acute AMR. Hence, antibiotic prophylactic therapy must be used judiciously at the discretion of the clinician.

Disclosure

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