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Hyperacute Antibody-mediated Rejection Associated With Red Blood Cell Antibodies

James Shaw, MD, PhD,[1] Ian W. Gibson, MD,[2] Chris Wiebe, MD,[1] Donald S. Houston, MD,[3] Joshua Koulack, MD,[4] David Rush, MD,[1] Peter Nickerson, MD,[1,5] and Julie Ho, MD[1,5]

The pathogenic role of non-HLA antibodies in antibody-mediated rejection (AMR) has been increasingly recognized, with inclusion in the Banff 2017 classification.[1] While several non-HLA antibodies have been implicated, it is clear there are additional uncharacterized non-HLA antigenic targets.[2] The minor histocompatibility antigens in the Kidd blood group system were first described in the 1950s,[3,4] and subsequent reports suggested that antibodies against Jk^a and Jk^b antigens can potentiate renal transplant rejection.[5-10] Jk^a and Jk^b are codominant alleles encoding a glycoprotein urea transporter expressed on the surface of red blood cells (RBCs), renal endothelium of the vasa recta, and tubular epithelial cells,[7,11,12] providing a plausible pathophysiological mechanism for Kidd-mediated AMR. We report a case of hyperacute rejection that we believe is best explained by preformed donor-specific anti-Jk^a antibodies.

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[1] Section of Nephrology, Department of Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[2] Department of Pathology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[3] Section of Hematology, Department of Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[4] Section of Vascular Surgery, Department of Surgery, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

[5] Department of Immunology, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

J.S., D.R., and J.H. drafted the case report, to which all authors subsequently contributed. I.W.G. provided transplant pathology expertise, including the histology figures and their interpretation. C.W. and P.N. provided HLA laboratory expertise, including the non-HLA antibody testing. D.S.H. provided hematological expertise on red blood cell antibodies, including their pathogenicity and evanescent nature. J.K. provided the detailed intraoperative description, which prompted the clinical diagnosis of hyperacute rejection.

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Correspondence: Julie Ho, MD, Departments of Internal Medicine & Immunology Sections of Nephrology & Biomedical Proteomics, University of Manitoba, GE421C Health Sciences Centre, 820 Sherbrook Street, Winnipeg, MB R3A 1R9, Canada. (jho@hsc.mb.ca).

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CASE DESCRIPTION

A 31-year male with end-stage renal disease secondary to IgA nephropathy on peritoneal dialysis for 3 years presented for deceased donor transplantation. He was blood group A, Rh positive, Jk^a negative, Jk^b positive, with anti-Jk^a antibodies, and calculated panel reactive antibodies 100% following 2 units RBC transfusion 3 years prior. Past medical history included beta-thalassemia trait, hypertension, and smoking. This out-of-province extended criteria donor (age >60 y and hypertension, kidney donor profile index 82) was matched via the Canadian highly sensitized patient registry with no pretransplant donor-specific antibodies (DSA). Flow crossmatch was negative with HLA mismatch 0A 1B 2DR 1DQ. Donor Kidd phenotype was unknown at this time. He received antithymocyte globulin (ATG) induction, methylprednisolone, mycophenolate mofetil, and tacrolimus. The transplant surgeon noted the donor renal artery was fragile and atherosclerotic. The surgery was complicated by recurrent intraoperative renal artery thrombosis during a 9-hour procedure involving reopening the arterial anastomosis to clear thrombus; redo of the anastomosis; repeat thrombus followed by arterial dissection; a saphenous vein patch; and redo anastomosis. Renal blood flow improved and was confirmed with intraoperative Doppler.

Unfortunately, the patient was anuric in the postanaesthetic care unit (PACU) and ultrasound demonstrated no blood flow, so he underwent same-day nephrectomy for graft thrombosis. Gross and histological examination revealed thrombosis, with ~70% luminal occlusion, affecting a major hilar vessel with the morphology of a vein. The main hilar renal artery appeared patent. There was no significant tubulointerstitial or microvascular inflammation, with Banff g0 i0 t0 v0 ptc0 and C4d negative in peritubular capillaries. There was no evidence of thrombotic microangiopathy. There was donor-related moderate arteriosclerosis and focal mild nodular hyaline arteriolosclerosis (Banff cv2 ah1) (Figure 1). At the time, primary nonfunction was attributed to recurrent platelet thrombus and the fragile nature of the artery. Hypercoagulable screen for anti-cardiolipin antibodies, lupus inhibitor, and beta2-glycoprotein was negative. Angiotensin receptor type II receptor antibodies were negative. The patient's recovery was unremarkable and immunosuppression was discontinued.

Seven weeks later, the patient received another deceased donor highly sensitized patient offer with no pretransplant DSA (Kidney donor profile index 41), HLA 0A 1B 1DR 1DQ mismatch, HLA eplet mismatch DRB1/3/4/5 14, and DQA1/

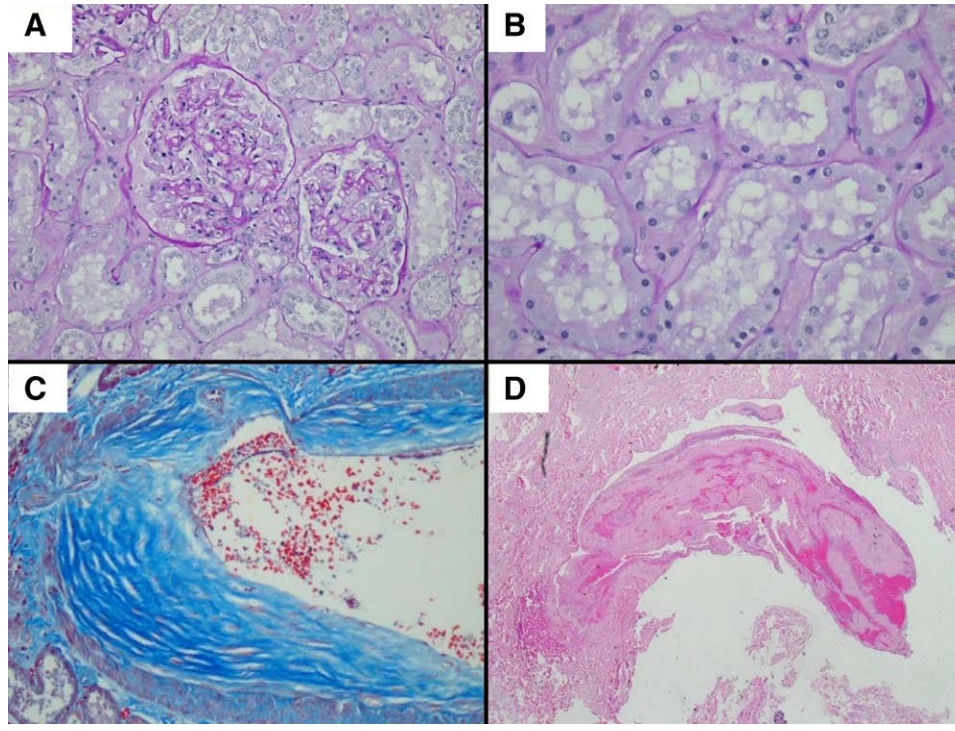

FIGURE 1. Transplant nephrectomy at <24 h posttransplant. No evidence of glomerulitis, tubulointerstital inflammation, or peritubular capillaritis (PAS, [A] ×200, [B] ×400). Moderate donor-related arteriosclerosis with intimal fibrosis (trichrome, [C] ×200). Renal hilar vessel with luminal thrombus (H&E, ×40).

DQB1 19. Flow crossmatch was negative, repeat hypercoagulable screen and angiotensin receptor type II receptor screen was negative. Donor Kidd phenotype was unknown at this time. Basiliximab induction and triple maintenance immunosuppression was used since he recently received ATG and was an Epstein Barr Virus mismatch (donor positive, recipient negative). Immediately after reperfusion, the kidney looked pink and healthy with immediate urine output. However, the graft became dusky within minutes of reperfusion, with decreased urine output and absent intraoperative Doppler flow. The anastomosis was taken down and platelet thrombus removed from the artery. Intravenous heparin, acetylsalicylic acid, and clopidogrel were administered while the anastomosis was redone. The graft appearance improved and operation completed.

In the PACU, he became anuric despite IV heparin infusion. Given the history of preformed anti-Jk^a antibodies with its expression on renal vascular endothelium and previous history of primary nonfunction from graft thrombosis, Jk^a was hypothesized as a donor-specific antigenic target. Donor Jk^a spleen typing confirmed the donor was Jk^a positive, and a presumptive diagnosis of anti-Jk^a hyperacute rejection was made. He was started on plasmapheresis within 2 hours of surgery in the PACU and preplasmapheresis serum was reactive with anti-Jk^a antibody titer 2. Jk^a is an evanescent RBC antibody, meaning that the titer can decrease over time,[13] and this titer is therefore considered grossly positive.

He received 5 daily plasmapheresis exchanges, with postplasmapheresis intravenous immunoglobulin (IVIg, 100 mg/kg/dose). He had an anaphylactic reaction to 1 unit fresh frozen plasma, so two-thirds of 5% human serum albumin and one-third of normal saline replacement fluid were used with plasmapheresis. His postoperative course was further complicated by a large retroperitoneal hemorrhage requiring 13 units Jk-matched RBC transfusion over the next several days, vasopressor support for hypotension, and renal replacement therapy for delayed graft function in the intensive care unit. Serial ultrasounds confirmed acceptable Doppler flow to the transplant.

After hemodynamic stabilization, anticoagulation reversal and holding dual antiplatelet therapy, a transplant biopsy was undertaken at 11 days. The sample was marginal, with 7 glomeruli and 1 small interlobular artery. There was neutrophilic glomerulitis (g2), focal severe peritubular capillaritis (ptc3) comprising neutrophils, and a significant number of CD68-positive mononuclear cells. There was acute tubular injury with regeneration. There was no evidence of tubulointerstitial inflammation (i0 t0), intimal arteritis (v0), or thrombotic microangiopathy. C4d was negative in peritubular capillaries. Immunofluorescence panel was negative for immunoglobulins, C3, C1q, and fibrinogen. Electron microscopy of a glomerulus showed scattered neutrophils and mononuclear cells in capillary lumina, with mild glomerular endothelial cell swelling, and focal mild glomerular epithelial cell foot process effacement. The diagnosis was C4d-negative AMR, based on the active microvascular inflammation (Figure 2). No donor-specific HLA antibodies were identified by solid phase assays. AMR was treated with IV ATG 4 mg/kg total over 3 days, methylprednisolone, 5 plasmapheresis, and IVIg treatments. By discharge, his anti-Jk^a antibodies were undetectable, and graft function stable with serum creatinine 447μmol/L.

He underwent serial anti-HLA antibody testing approximately every 10 days for the first 50 days. Solid phase single antigen bead assays were negative for DSA with the exception of a transient weakly positive anti-HLA-DQ7 DSA of unclear significance at postoperative day 39 (mean fluorescence intensity [MFI] 853) and 53 (MFI 710). This bead had been negative

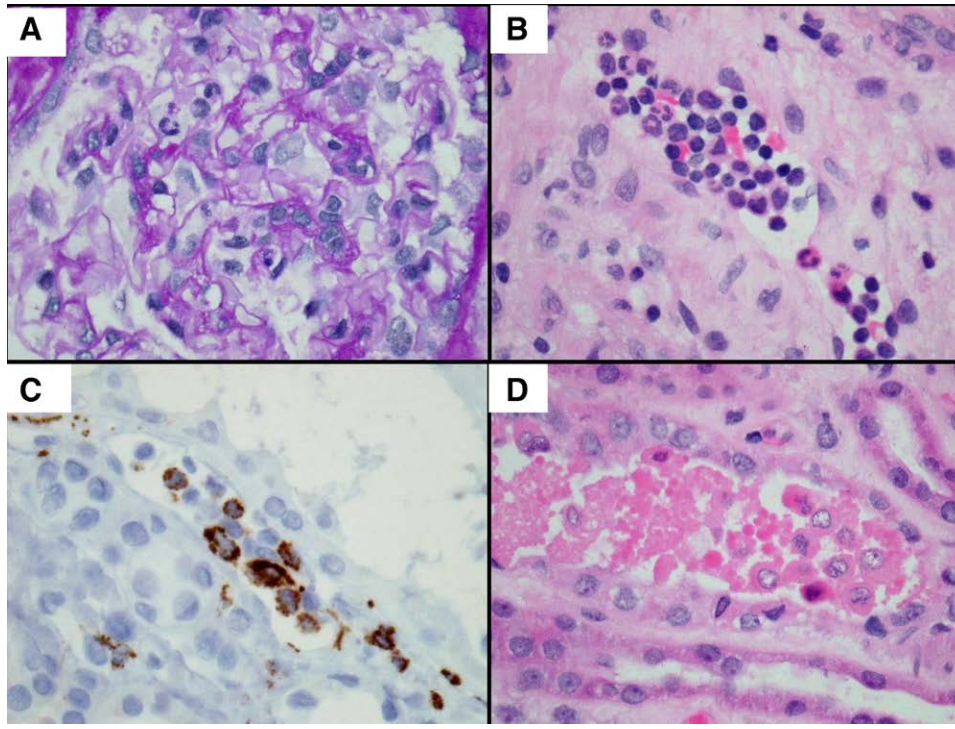

FIGURE 2. Eleven days posttransplant allograft biopsy. A, Neutrophilic transplant glomerulitis (PAS, ×400). B, Severe peritubular capillaritis with mononuclear cells and neutrophil polymorphs (H&E, ×400). C, CD68-positive mononuclear cells within peritubular capillaries (CD68, ×400). D, Acute tubular necrosis with sloughing of degenerate tubular epithelial cells (H&E, ×400).

at the time of transplant (MFI 145) and subsequently fell below 500 MFI by postoperative day 86. One-month biopsy was adequate with 21 glomeruli and 3 arteries (Figure 3A and B). There was ongoing active microvascular inflammation with mild neutrophilic glomerulitis and focal moderate peritubular capillaritis comprising mononuclear cells (g1 ptc2). Two glomeruli showed capillary loop fibrin thrombi. There was acute tubular epithelial injury with regeneration, and no significant tubulointerstitial inflammation or intimal arteritis (i0 t0 v0). Peritubular capillary C4d was negative. Immunofluorescence was negative for immunoglobulin, complement proteins, and fibrinogen. The diagnosis was continuing active C4d-negative AMR with glomerular thrombotic microangiopathy, treated with 5 plasmapheresis and IVIg treatments.

Three-month biopsy showed continuing active AMR with mild-to-moderate microvascular inflammation (g1 ptc2), patchy tubulointerstitial inflammation (i1 t2), and patchy interstitial fibrosis affecting ~60% of sampled cortex. C4d was negative in peritubular capillaries. Initial SV40 immunostain was focally equivocally positive in cortical tubular nuclei, suspicious for active polyomavirus nephropathy, but additional SV40 stains were negative. No increase in immunotherapy was undertaken due to the BK viremia and suspicion of polyomavirus nephropathy.

Anti-HLA-DQ7 was subsequently negative at 3 and 6 months posttransplant, and he had no other DSA. Ten-month biopsy showed resolution of active microvascular inflammation, and mild tubulointerstitial inflammation associated (Banff scores g0 i1 i-IFTA 2 t1 v0 ptc0). Features were suspicious for mild chronic active T cell–mediated rejection. One glomerulus of 11 sampled showed extensive focal segmental glomerulosclerosis, and electron microscopy showed patchy glomerular epithelial cell foot process effacement with areas of foot process preservation, consistent with secondary focal segmental glomerulosclerosis. There was patchy severe interstitial fibrosis/tubular atrophy affecting ~60% of sampled cortex (ci3 ct3) (Figure 3C and D). SV40 stain was negative. Peritubular capillary C4d was negative. Immunofluorescence panel was negative. The mild chronic active T cell–mediated rejection was treated with an oral prednisone pulse. Renal function stabilized at a creatinine 300–330 μmol/L, estimated glomerular filtration rate 22 mL/min/1.73 m^2, by 3 to 12 months posttransplant, and has slowly declined to creatinine 330–380 μmol/L, estimated glomerular filtration rate 17 mL/min/1.73 m^2 by 24 months posttransplant. The patient remains interested in a third transplant and will eventually undergo work-up to receive a Jk^a-negative renal transplant.

We sought to clarify the etiology of graft thrombosis and primary nonfunction in his first renal transplant. Retrospective analysis revealed the first donor was homozygous Jk^a/Jk^a positive, whereas the second donor was heterozygous Jk^a/Jk^b. We next sought to determine if preformed anti-Jk^a antibody is a more generalized cause of non-HLA AMR or primary nonfunction. Retrospective analysis of transplant recipients in our program, with microvascular inflammation (g+ptc ≥ 1 or isolated v-lesion) in the absence of a detectable DSA (n = 10) or primary nonfunction (n = 20), did not identify any additional patients with anti-Kidd or Duffy antibodies. Finally, a non-HLA antibody screen was performed to detect other potential causes of hyperacute AMR, but no strongly positive antibodies were identified. Notably, anti-perlecan (C-terminal fragment of perlecan) and anti-vimentin antibodies were negative (One Lambda LabScreen autoantibody groups 1–3).

DISCUSSION

Our patient experienced primary nonfunction due to recurrent renal artery thrombus with his first transplant, followed

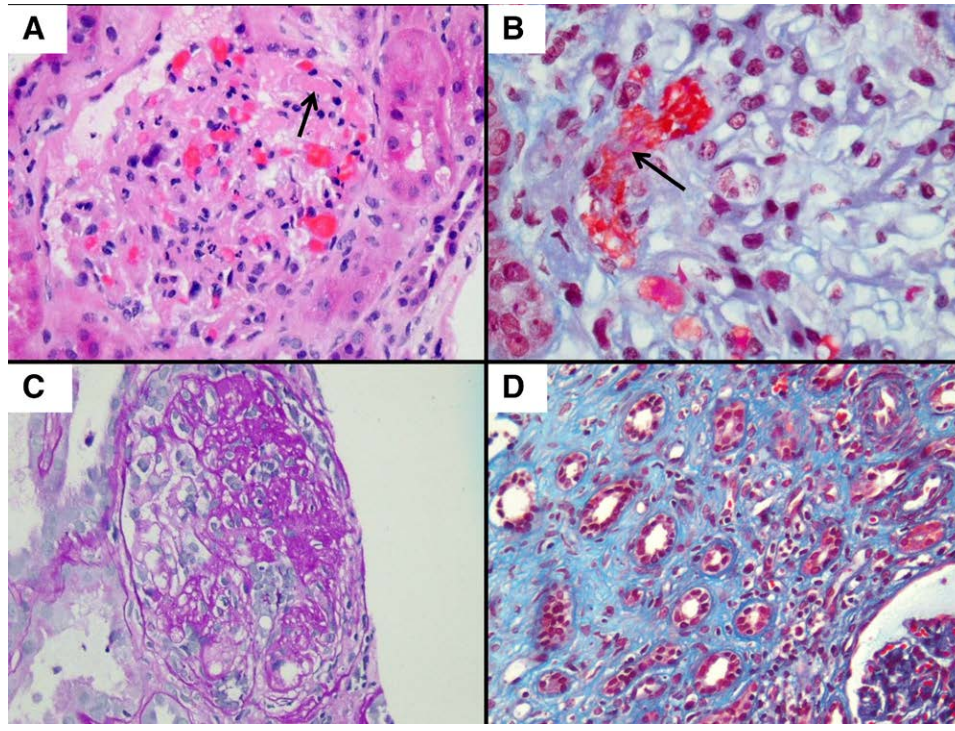

FIGURE 3. Three mo posttransplant allograft biopsy ([A] H&E ×200, [B] trichrome ×400). Neutrophilic transplant glomerulitis with focal glomerular capillary loop fibrin thrombi (arrows) 6 mo posttransplant allograft biopsy ([C] PAS, ×200, [D] trichrome, ×100). C, Focal segmental glomerulosclerosis. D, Interstitial fibrosis and tubular atrophy.

by hyperacute rejection with renal artery thrombus and microvascular inflammation during his second transplant. In both cases, there were preformed anti-Jk^a antibodies; Jk^a antigen on donor tissue; no anti-HLA DSA; negative non-HLA antibody screen; and no evidence of a hypercoagulable state. We speculate that anti-Jk^a–mediated hyperacute AMR best explains the immediate and recurrent donor renal artery thrombosis and delayed graft function. Notably, histological evidence of AMR was only present in the second transplant. In the first transplant, it is possible that increased homozygous Jk^a expression on renal vascular endothelium contributed to recurrent renal artery thrombosis, with inadequate perfusion reaching the graft to cause microvascular inflammation. This would be in keeping with the observation that anti-Jk^a reacts more strongly with Jk^a-homozygous RBC than heterozygous Jk^a/Jk^b RBC.[14] Conversely, other factors such as endothelial injury and poor donor artery friability may have also contributed to recurrent arterial thrombus and primary nonfunction of the first transplant. In the second transplant, the potential contributing role of the weak and transient DSA is acknowledged and cannot be definitively ruled out as an explanation for ongoing AMR. However, we argue that a Jk^a-mediated process is the likely cause of hyperacute AMR at the time of surgery and postoperative day 11 because the weak anti-HLA DSA was not detected until ~1 month posttransplant after IVIg had been administered, was never >853 MFI, and subsequently fell to <500 with serial testing. Finally, we cannot rule out the effects of other previously unidentified non-HLA antibodies.

Only a few cases of Kidd blood group-mediated AMR exist, with variable clinical presentations, but no hyperacute rejection. One early AMR occurred within a few days posttransplant in a patient with a history of blood transfusions and autologous bone marrow transplant. This appeared to be triggered by a transfusion reaction, and the authors speculate that passenger donor Jk^a-positive RBCs, plus transfusion, caused an immune stimulus that induced a transient memory anti-Jk^a response and severe AMR.[7] There are 2 reports of plasma cell-rich acute cellular rejection and one Banff 1A cellular rejection, with development of anti-Jk antibodies between 2 and 10 years posttransplant in nonadherent patients, which could have stimulated de novo donor-specific Jk antibody development.[5,6,8] Lastly, a case of C4d-positive AMR at 5 years posttransplant was reported with simultaneous anti-Jk^a antibodies resulting in graft loss.[9] Notably, our case was consistently C4d-negative on multiple biopsies, suggesting that anti- Jk^a antibodies do not always fix complement.

A retrospective study of 370 patients transplanted between 1991 and 1995 with >7-year follow-up showed that Jk-mismatched deceased donor-recipient pairs had more interstitial inflammation compared with matched controls, but the Jk-mismatch status had no influence on kidney allograft survival. Notably 37% transplants in that cohort had a Jk mismatch.[10] Therefore, many donor and recipient pairs are likely mismatched at the Kidd locus, and it is not fully understood why anti-Jk mismatch is pathological in some situations, but not others.

Our case is expected to be very uncommon. It is estimated that Jk^b-homozygous patients make an antibody to Jk^a-positive RBCs on 0.07% exposures.[15] Only 24% Caucasians are Jk^b-homozygous (eg, our patient), 26% Jk^a-homozygous (eg, first transplant), and 50% Jk^a/Jk^b–heterozygous (eg, second transplant).[14] Thus, this situation may arise in ~4–8 cases per 10 000 transplants in which the recipient has had prior red cell transfusion.

This case highlights the relevance of non-HLA antibodies during hyperacute rejection and the need to maintain a high

clinical suspicion for alternative causes of early graft thrombosis in the peritransplant setting. Anti-RBC antibodies, especially those with known cross-reactivity to renal tissue, should be identified to assist in immunological risk characterization. There is insufficient evidence to justify matching donor-recipient pairs at the Kidd locus, as this may preclude patients from receiving an otherwise compatible kidney, and anti-Jk AMR is a rare and poorly understood phenomenon. It is our hope this report will pique interest in further study of the Kidd blood group and its role in allograft rejection and prompt early consideration of Kidd-mediated hyperacute rejection as a potentially treatable cause of early graft thrombosis.

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