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# Eculizumab reverses the potentially fatal effects of kidney graft reperfusion injury

Kaabak M, Babenko N, Kuznetsov O, Matveev A, Minina M, Platova E, Morozova M, Novozhilova T. Eculizumab reverses the potentially fatal effects of kidney graft reperfusion injury.

Abstract: Half an hour after reperfusion, the kidney, transplanted to the infant from an adult brain dead standard criteria donor, became flabby and acquired blue color. Hyperacute rejection was suspected as a consequence of false negative cross match, and eculizumab was administered with the purpose to treat antibody-mediated injury, with fast and clear effect. The patient's blood was tested for donor-specific antibodies on the next day, and results were negative. We attribute graft damage to reperfusion injury and explain eculizumab's effectiveness to its ability to prevent progression of reperfusion injury.

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IRI is unavoidable during clinical organ transplantation. Severe IRI can result in graft primary non-function. It was generally accepted about 20 yr ago that any reperfusion injury would enhance HLA gene expression (1) and result in endothelial cell activation (2). However, current concepts of IRI indicate that it is more complex. In particular, it was recognized that IRI was mediated by complement activation via all three known pathways: lectin, classical, and alternative (3). Activation of terminal C5b-9 complement complex during kidney graft IRI in humans was shown by de Vries et al. (4).

It was hypothesized that IRI was a necessary trigger for allorecognition and rejection (5). Subsequently, Damman et al. (6) found systemic complement activation in deceased donors and demonstrated a direct association between C5b-9 level in donors and acute rejection by kidney recipients. Complement involvement was also demonstrated in IRI in both experimental and clinical heart transplantation, particularly when BD donors were involved (7).

Eculizumab is a monoclonal antibody that blocks the terminal components of complement activation. The safety and efficacy of eculizumab were initially established for treating paroxysmal nocturnal hemoglobinuria (8). It has also been used to prevent and treat atypical hemolytic uremic syndrome (9), anti-HLA-antibody-mediated kidney graft rejection (10), and antibody-mediated rejection during ABOi kidney–pancreas transplantation (11). However, eculizumab was found to be ineffective for preventing and

Abbreviations: BD, brain dead; IRI, ischemia–reperfusion injury; MPBI, multiplex bead-based immunoassay.

treating antibody-mediated rejection in two kidney graft recipients (12).

Here, we describe a case of catastrophic kidney reperfusion injury that was completely reversed following a single eculizumab infusion. We believe that our findings will encourage further investigations in this field.

### Case report

A 16-month-old boy with obstructive uropathy had been maintained on peritoneal dialysis since he was five months old. He received a right kidney transplant from a deceased 22-yr-old male who met standard donor criteria. This boy's serum had been screened for HLA IgG antibodies every three months during the waiting period using a MPBI; these results were negative. Complement-dependent lymphocytotoxic cross-matching with recipient blood taken two wk before transplantation was negative. Simple cold storage in Custodiol solution lasted 825 min, and the vascular anastomosis time was 24 min. Immunosuppression therapy included alemtuzumab (15 mg) administered subcutaneously two h before surgery, followed by tacrolimus monotherapy. Methylprednisone (125 mg) was administered once before reperfusion.

Thirty min after reperfusion, the kidney acquired an eggplant-like color and became flabby. Graft blood vessels appeared normal, although Hume test results were negative. Doppler ultrasound revealed an avascular cortex and weak blood flow in the segmental arteries with retrograde blood flow while in diastole (Fig. 1). Hyperacute rejection due to a false-negative cross-match was suspected, and eculizumab (300 mg) was administered IV. One h after eculizumab infusion, the turgor and color of the kidney returned to normal and Hume test results were positive.

The donor's spleen was requested from the organ procurement organization and was sent to our institution's laboratory along with recipient blood samples that were obtained just prior to surgery, before the eculizumab infusion, and post-surgery. Blood samples acquired in the operating room were tested for donor-specific anti-HLA antibodies using microbead-based assays. This assay included two types of beads that were conjugated with monoclonal antibodies specific for the HLA I and II classes. This assay also included control beads to monitor the assay background and to insure that the conjugates had been used. Donor lymphocytes were isolated from the spleen, lysed, and then incubated with these HLA class-specific beads. After incubation with recipient serum, anti-human IgG conjugated to phycoerythrin was added to the bead mixture. After washing, samples were analyzed using Luminex technology. All of the samples showed negative results.

Kidney graft function returned slowly. Urine appeared at six h after surgery and the creatinine level dropped to within the normal range within five days. A one-month surveillance biopsy revealed very weak tubular cell dystrophy and segmental basement membrane thickening in 2 of 10 glomeruli. No C4d deposits were detected. At 45 wk post-transplant, the boy had excellent graft function.

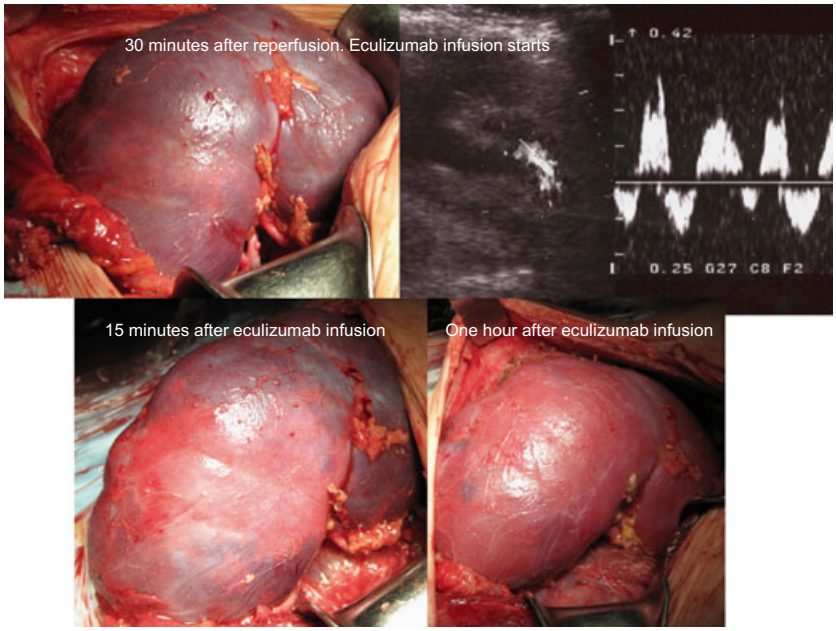


*Fig. 1.* Kidney graft appearance at 30, 45, and 90 min after graft reperfusion. Doppler ultrasound results when graft discoloration occurred.

## Discussion

The appearance of the kidney graft at 30 min after reperfusion was discouraging. Without effective intervention, cortical necrosis would have been unavoidable. At this time, a graft biopsy with no C4d deposits would be reliable evidence for the absence of antibodies. However, we did not perform this because anticoagulation therapy was anticipated for rejection treatment, and a diagnosis of hyperacute rejection seemed to be obvious. No other monoclonal or polyclonal antibodies or additional high-dose steroids were suggested as treatment options because this patient had received alemtuzumab, which targets both B and T cells. This patient's cell-mediated immunity was considered to have been sufficiently suppressed.

After graft discoloration occurred while in the operating room, we assumed that this phenomenon was due to preformed antigraft antibodies. Eculizumab was administered to block any complement-mediated toxicity. This was based on findings of Stegall et al. (10), who demonstrated that eculizumab could effectively prevent and treat antibody-mediated rejection phenomena.

Luminex bead technology is the most sensitive method for detecting anti-HLA antibodies (13). The negative MPBI results for all blood samples indicated that there were no anti-HLA antibodies both before and during transplantation; therefore, the compromised graft perfusion could not be explained by vascular rejection. In theory, non-HLA antibodies could cause this kind of damage. However, it did not seem likely that a non-sensitized infant would produce considerable amounts of antibodies. Additional evidence against a role for antibodies was the favorable post-operative course and the absence of C4d deposits on surveillance biopsy; however, alemtuzumab administered before transplantation could have affected B cells and prevented the further production of antibodies. Thus, reperfusion injury was the most plausible explanation for graft damage.

Other organs (heart, liver, second kidney, and pancreas) transplanted from the same donor who was brain dead due to cranial trauma exhibited excellent initial function. However, for our patient, reperfusion of the adult kidney occurred with a relatively low blood pressure. This patient's systolic pressure did not rise above 90 mm Hg with both volume intervention and dopamine infusion, and his mean systolic pressure after reperfusion was 72 mm Hg. We believe that hypotension was the cause for the severe graft injury in this case.

Although we could find no reports of IRI reversal with eculizumab treatment, the effect observed in this case was not surprising. Complement involvement in the development of IRI is undoubted and was thoroughly discussed in reviews by Damman et al. (14) and Chen et al. (3). Damman et al. (14) proposed that circulating complement components played a more prominent role in vascular endothelial damage after reperfusion of an ischemic kidney, whereas locally produced complement damaged other renal compartments. This was supported by findings by de Vries et al. (4), who found significant increases in soluble terminal complement complex in the blood of a graft vein during the first minutes after reperfusion.

For our patient, the successful reversal of kidney damage following eculizumab treatment suggests that complement plays a key role in the pathogenesis of reperfusion injury. Additional research will be necessary to clarify this suggestion. If the control of reperfusion injury by eculizumab can be confirmed, this would significantly affect current transplant practices from extending the conservation time to further decreases in rejection rates.

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## Disclosure

The authors have no conflict of interests to disclose.

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