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Clinical Report

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Acute antibody-mediated renal allograft rejection associated with HLA-Cw17 antibody

Manish Suneja and Sarat Kuppachi

Department of Internal Medicine, Division of Nephrology and Hypertension, University of Iowa Hospital and Clinics, IA, USA

Correspondence and offprint requests to: Manish Suneja; E-mail: manish-suneja@uiowa.edu

Abstract
Detection of donor-specific human leukocyte antigen (HLA) antibodies is an important part of diagnosis of antibody-mediated rejection (AMR) in the renal transplant population. Donor-specific antibodies (DSA) against HLA-C, a Class 1 major histocompatibility gene product, are not considered to be of major importance in renal transplant rejection. Typing for HLA-C is not a routine part of pre- and post-transplant evaluation. In roughly 10% of biopsy-proven C4d-positive rejections, DSA are not detected by standard testing protocols. In some of these cases, minor HLA and non-HLA antibodies have been implicated. The role of HLA-C antibodies in this patient group is not clear. We present a patient with acute renal graft dysfunction 21 months post-transplant. The allograft biopsy showed features of AMR with diffuse margination of inflammatory cells and diffuse C4d staining in peritubular capillaries. HLA-Cw17 antibody was detected by single-bead antigen Luminex assay, which was further confirmed by a mock flow crossmatch. This case highlights the importance of checking anti-HLA-Cw antibodies in patients with AMR and no detectable DSA using standard methods.

Keywords: antibody-mediated rejection; crossmatch; HLA-Cw17; kidney transplant; single-antigen bead assay

Introduction

Antibody-mediated rejection (AMR) is a significant cause of morbidity and mortality in the renal transplant population. AMR constitutes 20–30% of all cases of acute rejection. Donor-specific antibodies (DSA) are detected in 88–95% of cases of AMR in which diffuse peritubular capillaries (PTC) C4d is positive [1, 2]. In the remaining patients, non-human leukocyte antigen (HLA) and minor HLA antibodies may be implicated [3, 4]. HLA-C was identified in 1970 as a Class 1 gene product which could induce an alloantibody response [5]. Historically, HLA-C antibody was not considered to be an important factor in AMR, but its role in humoral sensitization is now recognized [6, 7]. In this context, we present a patient with biopsy features of AMR, who on additional testing had HLA-Cw17 DSA.

Case report

A 42-year-old male with end-stage renal disease due to focal segmental glomerulosclerosis received a single antigen-matched deceased kidney allograft transplant. Pre-transplant T- and B-cell complement-dependent cytotoxicity crossmatches were negative. He subsequently underwent induction with alemtuzumab and his immunosuppressive regimen included prednisone, cyclosporine and mycophenolate mofetil (MMF). His baseline serum creatinine (SCr) post-transplant varied from 1.6 to 2.0 mg/dL (122–153 μmol/L). Twenty-one months after the transplant, the patient presented to a local facility with complaints of malaise, fatigue and chest pain and was found to be in acute renal failure with an SCr of 3.2 mg/dL (244 μmol/L). Prior to this presentation, the patient had been lost to follow-up for 9 months. He was subsequently transferred to the university hospital for further evaluation. An acute cardiac process was ruled out. On admission, he was empirically started on high-dose steroids for concerns of cell-mediated rejection. On hospital Day 2, he underwent a renal allograft biopsy. The biopsy showed diffuse margination of neutrophils and mononuclear cells in the PTC. Mild interstitial inflammation and mild tubulitis were present. There was diffuse and bright staining for C4d in the PTC. The clinical and histological findings were consistent with an acute AMR as established by the Banff criteria [8, 9]. At the most recent Banff meeting, the role of C4d-negative AMR in kidney transplants was also acknowledged [10].

The recipient's and the donor's HLA phenotype are outlined in Table 1. The patient had no detectable DSA to HLA-A, -B and -DR using enzyme-linked immunosorbent assay on Day 1 of his hospital admission for acute renal failure. In light of the fact that renal biopsy was highly suggestive of AMR, additional testing using the Luminex® single-antigen bead (SAB) assay was performed (for Class I and Class II antigens) on the serum samples from Day 1 and Day 2 of the current admission. The DSA to HLA-A, -B, -DR and -DQ were negative. Antibodies against major histocompatibility complex Class I–related chain A (MICA) were negative. The only DSA detected using the Luminex® SAB was against HLA-Cw17 with a mean fluorescent intensity of 5500. For further confirmation, a mock flow

Table 1. HLA phenotype of the donor, recipient and mock donor[a]

Patient	A	B	Bw	Cw	DR	DRw	DQ
Recipient	2	8, 62	6	3, 7	4, 17	52, 53	2, 3
Donor	2, 66	27, 41	4, 6	2, 17	9, 13	52, 53	3, 3
Mock donor	3, 26	41, 56	7,10	1, 17	4, 4	53	ND

[a]ND, Not Done

crossmatch was done using harvested lymphocytes from a randomly selected HLA-Cw17-positive patient (see Table 1). This crossmatch was performed on three post-rejection serum samples from the patient (samples collected on Days 1, 2 and 5). All three post-rejection samples were positive for B-cell crossmatch but negative for T-cell crossmatch. In this mock crossmatch, specificity of the DSA was again confirmed to be against Cw17 antigen using Luminex® SAB.

The patient completed five sessions of plasmapheresis followed by intravenous immunoglobulin (IVIG) infusions as per our institution's protocol over a period of 14 days. His serum creatinine reached a peak of 4.1 mg/dL (313 μmol/L) 4 days after his hospitalization and subsequently returned to 2.7 mg/dL (206 μmol/L) after plasmapheresis and IVIG. After completing the treatment protocol, the patient's new baseline creatinine has remained 3.0–3.5 mg/dL (229–267 μmol/L) on an immunosuppressive regimen of prednisone, MMF and tacrolimus.

Discussion

Antibodies to major HLA Class I (A and B) and Class II (DR) are thought to be responsible for the majority of the cases of AMR. Minor HLA and non-HLA antibodies have been implicated in some cases of AMR [3, 4]. HLA-C DSA is not routinely checked in clinical practice and is not thought to be of importance in the pathogenesis of AMR in renal transplant patients [6]. The reason for this might be its low expression on the cell surface [11]. HLA-C-presented antigens, however, are recognized by cytotoxic T lymphocytes [12, 13]. In addition, an HLA-C locus has been shown to induce an antibody response similar to the other routinely checked loci [14]. The clinical significance of HLA-C antibodies has been unclear in kidney transplantation with only two reported cases of AMR caused by donor-directed HLA-C antibodies [15, 16]. Frohn *et al.* [17] in 2001 published a retrospective analysis, which suggested the influence of HLA-C mismatch in acute renal rejection. In this study, HLA-C mismatch significantly correlated with acute renal transplant rejection in pairs with one additional mismatch on the B locus. Influence of HLA-C has also been examined in bone marrow transplantation where it turns out to be a greater predictor of graft versus host disease compared to HLA-A and -B [18].

Earlier serological typing for HLA-C was considered less reliable than other HLA antigens [19]. Polymerase chain reaction kits are now commercially available, which can type the HLA-C loci accurately [20]. Also, with the availability of flow cytometric assays and antigen-coated bead assays, HLA-C antibodies can be identified with increased sensitivity which may allow us to elucidate their potential role in the pathogenesis of AMR.

In our patient, all three post-rejection serum samples had positive B-cell crossmatch, while T-cell crossmatch was negative. It could be that the B-cell crossmatch is more sensitive at detecting anti-HLA-C antibodies or it could be that non-HLA antibodies whose target antigens are expressed on B cells may have been involved. It is difficult to establish whether the DSA detected against the Cw17 antigen in this case were preformed or *de novo* as pre-transplant serum samples were not available for analysis.

This case highlights that in certain cases of AMR with no detectable DSA by standard testing, antibodies to non-typical antigens such as HLA-C may play an important role. Furthermore, it stresses the importance of including the HLA-C in donor typing and antibody analysis in sensitized prospective recipients.

Conflict of interest statement. None declared.

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