Clinical Case Report

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Successful kidney transplantation across a positive complement-dependent cytotoxicity crossmatch by using C1q assay-directed, bortezomib-assisted desensitization

A case report

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Abstract

Rationale: Human leukocyte antigen (HLA) is the major immunologic barrier in kidney transplantation (KT). Various desensitization protocols to overcome the HLA barrier have increased the opportunity for transplantation in sensitized patients. In addition, technological advances in solid-phase assays have permitted more comprehensive assessment of donor-specific antibodies. Although various desensitization therapies and immunologic techniques have been developed, the final transplantation decision is still based on the classic complement-dependent cytotoxicity (CDC) crossmatch (XM) technique. Some patients who fail to achieve negative XM have lost their transplant opportunities, even after receiving sufficient desensitization therapies.

Patient concerns: A 57-year-old male with end-stage renal disease secondary to chronic glomerulonephritis was scheduled to have a second transplant from his son, but CDC XM was positive.

Diagnoses: Initial CDC XM (Initial T-AHG 1:32) and flow-cytometry XM were positive. Anti-HLA-B59 donor specific antibody was detected by Luminex single antigen assay.

Interventions: Herein, we report a successful case of KT across a positive CDC XM (T-AHG 1:8 at the time of transplantation) by using C1q assay-directed, bortezomib-assisted desensitization. After confirming a negative conversion in the C1q donor-specific antibody, we decided to perform KT accepting a positive AHG-CDC XM of 1:8 at the time of transplantation.

Outcomes: The posttransplant course was uneventful and a protocol biopsy at 3 months showed no evidence of rejection. The patient had excellent graft function at 12 months posttransplant.

Lessons: The results of XM test and solid-phase assay should be interpreted in the context of the individual patient.

Abbreviations: AHG = anti-human globulin, CDC = complement-dependent cytotoxicity, DSA = donor-specific antibodies, DTT = dithiothreitol, HLA = human leukocyte antigen, IVIG = intravenous immunoglobulin, KT = kidney transplantation, MFI = mean fluorescence intensity, NIH = National Institute of Health, PP = plasmapheresis, XM = crossmatch.

Keywords: bortezomib, C1q-binding antibody, crossmatch, desensitization, donor-specific antibody, hyperacute rejection, kidney transplantation

1. Introduction

The presence of donor-specific antibodies (DSA) has been considered a contraindication for renal transplantation, as such its presence has been associated with hyperacute rejection and immediate graft loss.[1] A complement-dependent cytotoxicity (CDC) crossmatch (XM) was developed to identify DSA directed against donor lymphocytes, which are associated with hyperacute rejection. Consequently, various XM techniques were introduced to enhance test sensitivity. Furthermore, application of a solid-phase test for DSA has allowed detection and characterization of the relevant antibodies.[2,3]

With recent advances in immunologic tests, desensitization protocols to overcome the human leukocyte antigen (HLA) barrier have increased the opportunity for transplantation in sensitized patients with renal failure.[4,5] New interventions, such as bortezomib, are used for desensitization under the expectation of increasing transplantability.[6] However, decisions to perform kidney transplantation (KT) after desensitization were based on

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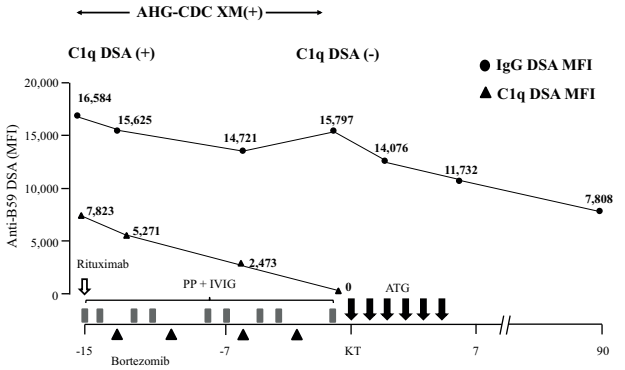

Figure 1. Desensitization protocol and trends in DSA. AHG = anti-human globulin, ATG = anti-thymocyte globulin, CDC XM = complement-dependent cytotoxicity crossmatch, DSA = donor-specific antibodies, IVIG = intravenous immunoglobulin, KT = kidney transplantation, MFI = mean fluorescent intensity, PP = plasmapheresis.

early experiences obtained prior to the introduction of solid-phase tests or new interventions.

In this study, we report a successful KT across a positive CDC XM (1:8 T anti-human globulin [AHG]) at the time of transplantation. After receiving a desensitization protocol comprising plasmapheresis (PP), intravenous immunoglobulin (IVIG), rituximab, and bortezomib, the patient's C1q-positive antibodies were converted to C1q-negative status.

2. Case review

A 57-year-old male with end-stage renal disease secondary to chronic glomerulonephritis underwent his first KT from his brother in 1996. He lost the allograft due to chronic rejection and returned to hemodialysis in 2011. He was scheduled to have a second transplant in another center from either his two sons or his wife, but all were CDC XM positive. A desensitization protocol using high-dose IVIG failed to achieve a negative CDC XM with his donors. Subsequently, he was put on the waiting list for 4 years and offered kidneys twice. However, neither time he could undergo KT because he revealed positive CDC XM reactions to the 2 deceased donors. He was then referred to our center for second living donor (from his older son) KT.

The donor and recipient were mismatched on 2 HLA loci (donor A2,33; B58,59; DR4,13 and recipient A33,33; B46,58; DR4,13). Initial CDC XM (National Institute of Health [NIH]-T cell-CDC XM 1:1 and AHG-T cell-CDC XM 1:32) and flow-cytometry XM were positive. Dithiothreitol (DTT) treated patient sera (final DTT concentration of 0.005 M) were used for CDC and flow-cytometry XM. Pronase-treated donor lymphocytes were used for flow-cytometry XM. Anti-HLA-B59 DSA was detected by Luminex single antigen assay (LABScreen SAB Class I and Class II; One Lambda, Canoga Park, CA) with a mean fluorescence intensity (MFI) of 16,584 and by C1q assay. His calculated panel reactive antibody with levels above 1000 MFI was 97.9%. We planned a desensitization protocol comprising a single dose of rituximab (375 mg/m^2), PP followed by IVIG (100 mg/kg/d), and bortezomib (Fig. 1). Tacrolimus (target trough level: 3–8 ng/mL) was started 1 week before transplantation. Induction therapy with anti-thymocyte globulin at a dose of 1.5 mg/kg/d was initiated at operation day and continued for 5 days after transplantation.

After 2 sessions of PP + IVIG, the MFI value of the DSA was still >10,000 and the C1q DSA result was positive. The patient received 1 cycle of bortezomib (1.3 mg/m^2, on days 1, 4, 8, and 11) along with further PP + IVIG treatment. Although the MFI value of the DSA remained over 10,000, the C1q DSA result became negative. Changes in antibodies against donor-specific epitopes are presented in Table 1. Three-dimensional HLA models demonstrated that desensitization had a different effect on epitope reaction between IgG and C1q assays (Fig. 2).

After confirming a negative C1q-binding DSA, we decided to perform KT accepting a positive AHG-CDC XM of 1:8 at the time of transplantation. The kidney appeared well perfused and did not show evidence of hyperacute rejection.

The patient did not receive posttransplant PP + IVIG treatment, and his postoperative course was uneventful. The protocol biopsy performed at postoperative month 3 showed mild tubular dilatation with a few casts. Peritubular C4d staining was negative

Table 1

Changes in SABA IgG and SABA C1q assays after desensitization.

Informative epitopes based on donor-specific epitope reaction*	Pre-desensitization		Post-desensitization	
	SABA IgG	SABA C1q	SABA IgG	SABA C1q
69TNT	Positive	Positive	Positive	Negative
66IF+163TEW	Positive	Positive	Positive	Negative
80I+65QI	Positive	Positive	Positive	Positive
131s+163T	Positive	Positive	Positive	Negative
163TEW+65QI	Positive	Positive	Positive	Negative
45EE	Positive	Positive	Positive	Negative
62RNQ	Positive	Negative	Positive	Negative
66IF	Positive	Positive	Positive	Positive
71TTN	Positive	Positive	Positive	Negative
94TW	Positive	Positive	Positive	Positive
97T	Positive	Positive	Positive	Positive
113HN	Positive	Positive	Positive	Positive
116L	Negative	Positive	Negative	Negative
NIH-CDC-XM	Positive (1:1)		Negative	
AHG-CDC-XM	Positive (1:32)		Positive (1:8)	

AHG = anti-human globulin, CDC = complement-dependent cytotoxicity, NIH = National Institute of Health, SABA = single antigen-bead assay, XM = crossmatch.

* Epitope reactions were analyzed using HLA matchmaker (HLA-ABC antibody analysis [v02] updated on June 2016, http://www.epitopes.net/) and informative epitopes are defined based on HLA epitope registry (http://www.epregistry.ufpi.br/).

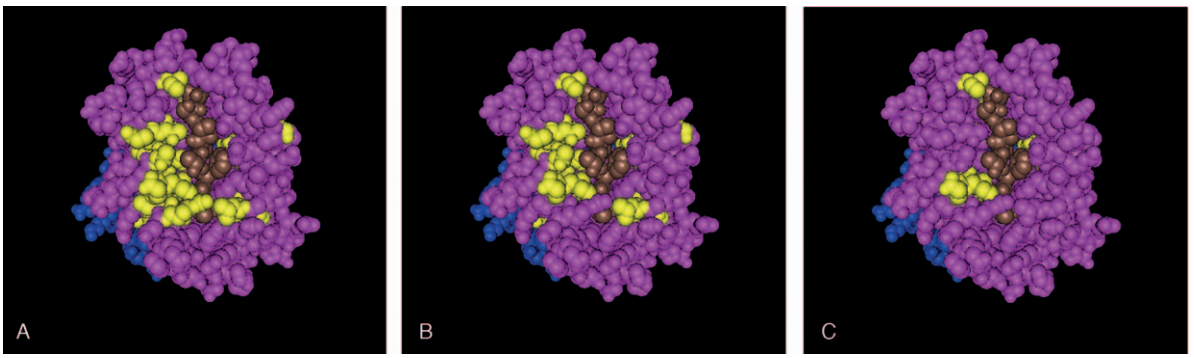

Figure 2. Three-dimensional HLA models with immunogenic epitopes. (A) Multiple donor-specific epitope reactions were positive in IgG assay at pre- and post-desensitization. (B) Donor-specific epitope reactions in the C1q assay prior to desensitization. (C) Donor-specific epitope reactions in the C1q assay after desensitization. Pink: alpha domain, blue: beta domain, brown: peptide, yellow: reactive donor-specific epitopes. Residue locations defining epitopes on the HLA molecular surface were visualized using the Cn3D structure and sequence alignment software program (downloaded from https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.html).

under immunofluorescence, and no endothelial swelling or peritubular capillary changes were present on examination via electron microscopy. Additional kidney injury molecule-1 immunohistochemistry for the evaluation of acute tubular injury showed negative staining in proximal tubules (Fig. 3). Currently, at 12 months post-transplantation, the patient continues to do well with a baseline creatinine level of 1.0 mg/dL and a urinary protein-to-creatinine ratio of less than 0.1 (g/gCr). Anti-HLA-B59 DSA remains at MFI 7815, but no HLA antibody reaction has been detected by C1q assay. The patient did not suffer acute rejection or infectious complication during follow-up.

The study procedures were in accordance with the Declaration of Helsinki. All procedures were performed after obtaining informed consent. A single patient case report does not require institutional review board approval according to our board policy.

3. Discussion

Historically, several cases of transplantation with a positive CDC XM at transplantation were performed in the past prior to the introduction of solid-phase tests or new interventions.[4,7] In those cases, most patients had poor outcomes, but some cases did have acceptable outcomes. The reasons for the different courses remain unknown, but a positive CDC XM is a contraindication to transplantation. Afterward, despite advances in desensitization protocols and immunologic testing, most KTs are performed after achievement of a negative CDC XM. In this report, we present a successful case of KT with a persistently positive CDC XM (T-AHG 1:8 at the time of transplantation) following C1q assay-directed, bortezomib-assisted desensitization.

KT is the treatment of choice for end-stage renal disease patients. However, over 30% of patients on a waiting list are sensitized to HLA due to pregnancy, blood transfusion, or previous transplantation. These sensitized patients are less likely to find compatible deceased donors and thus have a prolonged waiting time. In addition, they have a limited possibility of undergoing a paired kidney exchange.[8] Desensitization protocols to overcome the HLA barrier have become increasingly popular because of organ shortages and have increased the opportunity for transplantation in sensitized patients.[4,5,9]

Despite a low incidence of hyperacute rejection and acceptable short-term outcomes in sensitized patients, antibody-mediated rejection remains a significant challenge even after successful desensitization.[10] Concern that relevant DSA are not being detected by less sensitive assays has led to the development of more sensitive XM techniques. Paradoxically, CDC XM has been used as a minimum requirement for KT due to its low sensitivity.[11] However, recent studies have demonstrated that KT recipients from HLA incompatible donors exhibit a substantial survival benefit compared to that in patients who did not undergo transplantation and those who waited for

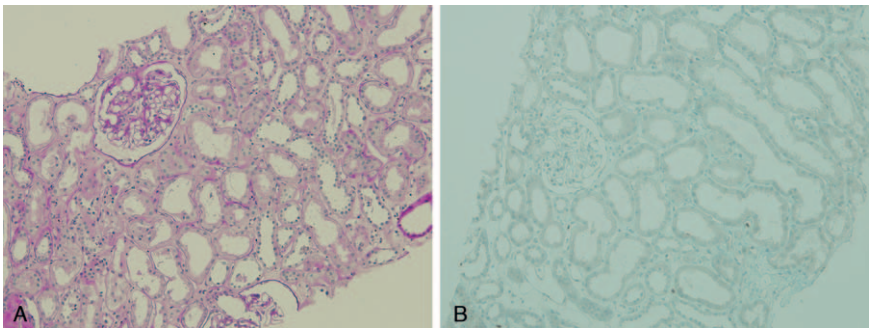

Figure 3. Protocol biopsy. (A) Renal structure is well preserved without glomerulitis or tubulointerstitial inflammation (Periodic acid-Schiff, ×100). (B) Tubules are intact and do not show positivity with kidney injury molecule-1 immunohistochemical staining (KIM-1, ×100).

transplants from deceased donors, regardless of DSA levels.[12] Therefore, accurate analysis of an individual's DSA, which helps in the decision on whether to proceed with a transplantation, is critical for a patient.

Although the development of solid-phase assays has greatly elucidated the role of DSA in the immune response to allografts, it is difficult to determine an MFI cutoff value suitable for proceeding to transplantation.[13] In fact, MFI values determined with conventional solid-phase assays do not accurately correlate with the complement-binding DSA. In contrast, the C1q assay directly discriminates the ability of a particular complement-binding DSA. It is quite important in a clinical setting considering that the DSA mediated complement cascade is the hallmark of in vivo graft injury.[14] The classic CDC XM is neither sensitive nor specific enough to detect complement fixation.[15,16] In our case, the AHG-CDC-XM result was 1:8 positive, but the NIH-CDC-XM and C1q DSA results were negative at KT. Hence, we decided to perform KT considering not only the CDC XM indicators, but also the results of the solid-phase tests. To the best of our knowledge, this is the first report of successful KT following C1q assay-directed desensitization.

Desensitization protocols have been based on PP and/or IVIG, which physically remove or inhibit the circulating DSA. In addition, rituximab is widely used for reducing B-cells.[4,9] Nevertheless, prior desensitization protocols have demonstrated varying degrees of efficacy and durability. A potential explanation for the variation among results is the failure to inhibit plasma cells, the source of antibodies.[6]

Bortezomib, a proteasome inhibitor, exerts an inhibitory effect directly on antibody-secreting plasma cells. Prior study using bortezomib-based desensitization represented a significant and sustained reduction in DSA, which may allow for increased transplantability.[6] We achieved a negative C1q conversion in this highly sensitized patient by using bortezomib as an additional therapy. In addition, the patient did not suffer clinical rejection in the absence of postoperative PP and IVIG. The protocol biopsy also demonstrated no evidence of subclinical rejection.

Interestingly, the patient showed positive AHG-CDC XM without C1q-binding DSA. Therapeutic agents used in desensitization protocols have been shown to cause interference in XM results.[17] In our case, however, the positive CDC XM is hard to be deemed a false positive considering the high MFI of DSA at the time of transplantation. Rather, epitope analysis can provide a possible explanation for the positive CDC XM reaction. Epitopes are parts of an antigen recognized by antibodies. As epitope reaction depends on the 3-dimensional structure, changes in this structure may lead to changes in the antibody reactivity.[18] If there is an insufficient conformational area due to a structural change, even though some C1q epitope reactions were positive, but overall C1q DSA could be negative. After the desensitization, the HLA-B59 DSA itself turned into negative in the C1q assay, but multiple donor-specific epitope reactions remained positive in the IgG assay with several epitope reactions in the C1q assay. These complement-binding antibodies against donor-specific epitopes with high concentrations of noncomplement-binding antibodies might induce a positive AHG-CDC XM reaction even after desensitization.

In conclusion, we report a successful KT across a positive CDC XM at the time of transplantation, by using C1q assay-directed, bortezomib-assisted desensitization. The goal of desensitization is to maximize the opportunity for transplantation, with improved mortality and quality of life compared with that associated with dialysis. However, for over 40 years, transplantation decision making has been based on the CDC XM, regardless of the presence of more sensitive and more accurate immunologic tests. The cost of continuing the historical approach is that of eliminating some patients that could have undergone a successful transplant. Therefore, the results of XM tests and solid-phase assays must be interpreted in the context of the individual patient.

References

[1] Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735–9.

[2] Gloor JM, Winters JL, Cornell LD, et al. Baseline donor-specific antibody levels and outcomes in positive crossmatch kidney transplantation. Am J Transplant 2010;10:582–9.

[3] Konvalinka A, Tinckam K. Utility of HLA antibody testing in kidney transplantation. J Am Soc Nephrol 2015;26:1489–502.

[4] Stegall MD, Gloor J, Winters JL, et al. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. Am J Transplant 2006;6:346–51.

[5] Vo AA, Lukovsky M, Toyoda M, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. N Engl J Med 2008;359:242–51.

[6] Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. Am J Transplant 2015;15:101–18.

[7] Zachary AA, Montgomery RA, Ratner LE, et al. Specific and durable elimination of antibody to donor HLA antigens in renal-transplant patients. Transplantation 2003;76:1519–25.

[8] Gentry SE, Segev DL, Montgomery RA. A comparison of populations served by kidney paired donation and list paired donation. Am J Transplant 2005;5:1914–21.

[9] Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-incompatible kidney recipients and survival. N Engl J Med 2011;365:318–26.

[10] Orandi BJ, Garonzik-Wang JM, Massie AB, et al. Quantifying the risk of incompatible kidney transplantation: a multicenter study. Am J Transplant 2014;14:1573–80.

[11] Gloor J, Stegall MD. Sensitized renal transplant recipients: current protocols and future directions. Nat Rev Nephrol 2010;6:297–306.

[12] Orandi BJ, Luo X, Massie AB, et al. Survival benefit with kidney transplants from HLA-incompatible live donors. N Engl J Med 2016;374:940–50.

[13] Reinsmoen NL, Lai CH, Vo A, et al. Acceptable donor-specific antibody levels allowing for successful deceased and living donor kidney transplantation after desensitization therapy. Transplantation 2008;86:820–5.

[14] Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215–26.

[15] South AM, Grimm PC. Transplant immuno-diagnostics: crossmatch and antigen detection. Pediatr Nephrol 2016;31:897–905.

[16] Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol 2011;72:849–58.

[17] Milongo D, Vieu G, Blavy S, et al. Interference of therapeutic antibodies used in desensitization protocols on lymphocytotoxicity crossmatch results. Transpl Immunol 2015;32:151–5.

[18] Duquesnoy RJ, Marrari M, Jelenik L, et al. Structural aspects of HLA class I epitopes reacting with human monoclonal antibodies in Ig-binding, C1q-binding and lymphocytotoxicity assays. Hum Immunol 2013;74:1271–9.