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Case Report

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De-novo donor specific DQ antibody in AMR and need for extended HLA typing

Abstract

Introduction: Renal transplant workup has evolved tremendously from Complement Dependent Cytotoxicity (CDC) crossmatch to more sensitive tests like Flow-cytometry crossmatch (FCXM) and Luminex based crossmatch and Single Antigen Bead (SAB) assay. Post-transplant de-novo antibodies particularly DQ have been reported as cause of antibody mediated rejection (AMR).We present two cases of End stage renal disease (ESRD) patients awaiting second renal transplant where extended HLA typing of donor became crucial to confirm donor specific de novo DQ antibodies.

Case reports: We present two cases of un-sensitized patients who underwent renal transplant, following which they developed acute AMR. Patient 1, was worked up for a 2nd transplant and a SAB assay was performed. The SAB assay was positive for Class II antibodies. Antibodies were present for only two beads, DQA1*02:01-DQB1*06:01 and DQA1*03:02-DQB1*03:03. HLA-DQ typing for the first donor confirmed DQA1*03 and DQB1*06 as de novo donor specific antibodies (DSA). For patient 2 patient, AMR was suspected 2 years post transplant. A SAB assay was done to confirm diagnosis and it was positive for a single bead. Extended typing of the donor confirmed DQA1*05/DQB1*03 as DSA with Mean Fluorescence Intensity (MFI) of 21,036.

Conclusion: Despite the advances in transplantation, de novo HLA antibodies continues to be a major hurdle, which can go unnoticed due to limited HLA typing. These cases favour the need for extended HLA typing (DQ) to closely monitor and prognosticate alloantibody formation and to initiate possible desensitization.

Keywords: alloantibody, de novo antibody, luminex, single antigen bead, transplant

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Abbreviations: CDC, complement dependent cytotoxicity; FCXM, flow-cytometry crossmatch; SAB, single antigen bead; ESRD, end stage renal disease; AMR, antibody mediated rejection; DSA, donor specific antibodies; MFI, mean fluorescence intensity; DSA, donor specific antibodies; HLA, human leukocyte antigens; PRA, panel reactive antibody; MCS, median channel shift; AMR, antibody mediated rejection; PE, phycoerythrin conjugate; IVIG, intravenous IG

Introduction

Alloantibodies in renal transplant scenario have been categorized as

i. Complement binding and non-complement binding

ii. Anti-HLA and non HLA

iii. Donor specific antibodies (DSA) and Non donor specific

To identify these various methods have evolved over time, namely Complement Dependent Cytotoxicity (CDC) to more sensitive tests such as Flow-cytomtery and the Luminex platform.[1–3] All these methods have their merits and demerits. While the CDC has high specificity for detecting complement binding antibodies, it has low sensitivity. The Flow cytometry crossmatch is donor specific, but cannot discriminate non-HLA IgG antibodies. Lastly the Luminex platform is a very sensitive assay and identifies single anti-HLA specificity; however it has its limitations such as confounding effect due to prozone phenomena and defining the threshold value for tests in each laboratory.[4] All these methods are used together as part of renal transplant work-up. Despite these advances, allograft dysfunction remains a significant problem.

De novo formation of antibodies against donor human leukocyte antigens (HLA) has been recognized as one of the major risk factors for reduced allograft survival. De novo antibodies have been described as those which were not present or identified in a patient previous to transplant, but detected post transplant, and have been reported to be as frequent as 15-25% in 5years post transplant patients.[5] These antibodies have been reported towards both Class I and Class II antigens, however more frequently commonly towards the latter, particularly DQ for which HLA typing is not routinely performed.[6–8]

As per international guidelines laboratories must be able to identify antibodies to HLA A, B, C, DP, DR and DQ and to further indentify DSA.[9] However in present scenario in developed countries such as United States with high proportion of cadaveric transplant most allocation algorithms match for HLA-A, B and DR and most transplant societies of developing countries like India where live related transplant are the major proportion also consider HLA A, -B and -DR loci alone and not the extended typing involving HLA-C, DP (DPA1 and DPB1) and DQ (DQA1 and DQB1).[10–12]

We hereby present two cases awaiting second renal transplant wherein de-novo DQ antibodies were identified and extended HLA typing became crucial prior to the second transplant.

Case 1: A forty year old male underwent renal transplant with a related donor. The patient had no sensitization history and CDC, FCXM for T and B cells and panel reactive antibody (PRA) were all negative. The Flow-crossmatch was performed using 0.25million donors isolated peripheral blood mononuclear cells suspended in 50µl of media mixed with 50µl of test serum and incubated for 30minutes at 4°C. Then the cells were washed with cold media. A 50µl of Goat-Anti Human IgG (Jackson ImmunoResearch Laboratories inc., USA), 10µl of Anti-CD22-PE (Beckton Dickinson, USA) and Anti CD3-PerCp (Beckton Dickinson, USA) were added to the cell pellet and incubated for 30minutes at 4°C. Then the cells were washed and re-suspended in 500µl of PBS. The lymphocytes were gated on FSC-SSC dot plot. These gated lymphocytes were then resolved into T and B-cells using CD3 and CD22 dot plot respectively. Each of these populations was gated and the MFI of these was evaluated for IgG FITC. A median channel shift (MCS) of 50 and 80 for T and B cells respectively, beyond the negative control value, were used to call a test as positive.

The patient received six units of blood transfusion during surgery. Following transplant patient developed allograft dysfunction after four months and antibody mediated rejection (AMR) was confirmed on biopsy. The historical serum was re-tested to rule out possibility of a preformed antibody. Patient presented two years later awaiting second renal transplant, with his sister as the probable donor. In view of the risk associated, a SAB was done in addition to the CDC and FCXM.

The SAB assay is a qualitative detection method for IgG anti HLA antibodies using the Lifecodes LSA Class-I & Class-II kits, using Luminex®. (Immucor Transpalnt diagnostics, inc, Stanford, CT, USA). The recipient serum is incubated with beads coated with recombinant HLA antigens and after removing excess serum antibodies by wash buffer, an anti-human IgG phycoerythrin conjugate (PE) is added. Acquisition of the beads is done on Luminex using Xponent software. The analysis is done using Xponent match IT antibody software. The signal intensity for each bead is compared to the signal intensity of negative control beads included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

The SAB assay was negative for Class I however showed positivity for Class II anti-HLA antibodies. Antibodies were identified against only two beads, DQA1*02:01-DQB1*06:01 and DQA1*03:02-DQB1*03:03 with mean fluorescence intensity (MFI) values of 8823 and 1500 respectively. The CDC and flow crossmatch were still negative. In view of history of AMR during previous transplant, an extended HLA typing was done for the first donor. HLA-DQ typing confirmed these as de-novo antibodies and DQA1*03 and DQB1*06 were confirmed as donor specific antibodies (DSA). However these were non DSA for the second donor (Table1).

Table 1 Case 1-HLA typing (low resolution for patient and both donors)

	HLA –A	HLA-B	HLA DRB1	HLA DQA1	HLA DQB1
Patient	A*01, A*02	B*08, B*08	DRB1*03, DRB1*03	-	-
1st Donor	A*24, A*68	B*08, B*51	DRB1*04, DRB1*13	DQA1*01, DQA1*03	DQB1*06, DQB1*06
2nd Donor	A*02, A*02	B*08, B*40	DRB1*03, DRB1*14	DQA1*01, DQA1*05	DQB1*02, DQB1*05

Case 2: A thirty one year old nulliparous lady, with no history of transfusion underwent renal transplant with her mother being the donor following a negative CDC, FCXM and PRA. The post transplant period was uneventful. However two years post transplant she was worked up due to increasing creatinine level trend and renal biopsy revealed, acute AMR and SAB assay was performed. SAB was negative for Class I, however positive for Class II with antibodies towards only DQ antibodies. In view of suspected rejection, extended typing was done for the donor and DQA1*05/DQB1*03 were identified as donor specific antibodies (DSA) with MFI of 21,036. Cascade plasmapheresis was immediately initiated and she was put on the waiting list for a second transplant (Table 2).

Table 2 Case 2-HLA typing (low resolution for patient and donor)

	HLA –A	HLA-B	HLA DRB1	HLA DQA1	HLA DQB1
Patient	A*01, A*02	B*08, B*08	DRB1*03,DRB1*03	-	-
1st Donor	A*01, A*01	B*08, B*27	DRB1*03, DRB1*11	DQA1*05, DQA1*05	DQB1*02, DQB1*03

Discussion

HLA Class II molecules are being studied extensively for both the matching outcome in transplant cases and also for antibodies to these antigens. While the Class I and Class II antigens are similar in most aspects, one crucial difference is the fact that while Class I molecules are formed by the peptide-binding groove in a single protein chain, the class II molecules are formed from two structurally homologous α- and β-chains that each contributes half of the peptide-binding groove. The HLA DQ molecule is formed from two chains of alpha and beta each; two alpha protein domains, coded by the DQA1 gene, and two beta protein domains, coded by the DQB1gene. The HLA-DQ antigen typing is determined by HLA-DQB1 genotyping. HLA-DQ antigen Luminex SAB beads are coated for both DQ alpha and DQ beta proteins. Therefore, in antibody screening as opposed to antigen typing DQ alpha proteins are also are taken into account for the interpretation.[13]

The two cases discussed above highlight the need for extended HLA typing in renal transplant cases and help understand the role of de novo anti-HLA DSA in renal transplant outcome. Detection of alloantibodies in previously un-sensitized patients post transplant has been studied extensively. De novo antibodies and particularly towards DQ loci have been found to cause both acute and chronic rejection and have been associated with poor graft outcome.[7,14–16] Therefore in the era of minimal immunosuppression it has become even more important to identify de novo antibodies early and manage these patients accordingly.

In the last one and a half years our laboratory has performed 67 SAB assays and sixteen of these patients are patients awaiting a

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second transplant. Of these 67patients, 40% have been found positive for either class I, II or both antibodies. The present two cases are representative of formation of de-novo DQ antibodies. De novo DSA have been reported towards both Class I and Class II HLA antigens, however found to be predominantly towards Class II,[7,8] however there are few studies that have reported to the contrary.[17] Among Class II antibodies, DQ antigens have been found to be most frequent.[8] Incidence of de novo antibodies has been reported varying from 5.5% to 32% (18-19). Hourmant et al.[18] have further reported 98% to be due to Class II. While Alberu et al.[19] found 7.5% of 32% towards Class II.[18,19] De-novo antibodies have been associated commonly with chronic rejection; however there are case reports of acute AMR due to de-novo antibodies particularly towards DQ.[14]

Studies have reported correlation between presence of donor-specific anti-HLA-DQ and risk for transplant rejection.[5–7,14–16] There is literature to suggest that there is a high frequency of antibodies formed to DQ antigens post transplant due to the highly polymorphic nature if the genes encoding for the molecule. Hence sensitizing events lead to formation of DQ antibodies by the immune system.[20] Interpretation of DQ antibodies has been extensively discussed by Haarberg et al.[10] suggesting that since HLA class II antigens are composed of two chains, α and β it is important to consider the contribution of both while analyzing antibodies rather that beta chain alone, as has been common practice.[10] This practice was due to the fact that the alpha chain of DR is virtually non-polymorphic, however it is not so for DQ and DP which have polymorphic alpha chain and contributes to alloantibodies. Therefore when anti-HLA DQ and DP are being assessed the role of alpha chain has to be considered and SAB assay interpreted with caution.[10] Therefore this finding emphasizes that extended typing for DQA1, DQB1, DPA1 and DPB1 are essential.

Post transplant monitoring is recommended at regular intervals of three months during first year followed by annual testing. This monitoring is intended towards identifying early signs of graft dysfunction and help pick up de novo antibodies. However this is not always adhered to due to economic constraints. In present trend of treatment post transplant where clinicians aim at reducing immunosuppressant gradually over the course of time, it becomes even more critical to identify any new antibodies which might be coming up. Therapeutic strategies, including combinations of plasmapheresis (or immunoadsorption), intravenous Ig (IVIG), and Rituximab (anti-CD20), along with tacrolimus and mycophenolate mofetil, have been used successfully to treat rejection.[6]

Conclusion

In our opinion extended HLA typing should be done routinely for all patients awaiting transplant to monitor them more closely for antibodies to all loci and also overcome any hurdles for risk prediction during second transplant. Secondly, de novo antibodies especially towards DQ can cause both acute and chronic rejection and should not be ignored. Early identification of these underlying de novo antibodies can help clinicians offer solutions such as plasmapheresis to the patient and improve the post transplant course.

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Conflict of interest

The author declares no conflict of interest.

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