CASE REPORT

Positive flow cytometry crossmatch with discrepant antibody testing results following COVID-19 vaccination

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Abstract

The impact of COVID-19 vaccination on the alloimmunity of transplant candidates is unknown. We report a case of positive B cell flow cytometry crossmatch in a patient waiting for second kidney transplantation, 37 days after receiving the COVID-19 vaccine. The preliminary crossmatch, using sample collected before COVID-19 vaccination, was negative. The antibodies to mismatched donor HLA-DR7 were detected only with multi-antigen beads but not with single-antigen beads, excluding possible prozone effects in solid-phase antibody assays. The crossmatches were positive with HLA-DR7–positive surrogates ($n = 2$) while negative with HLA-DR7–negative surrogates ($n = 3$), which confirms the HLA-DR7 alloreactivity. The antigen configurations on B lymphocytes are similar to that on the multi-antigen beads while distinct from the single-antigen beads. HLA-DR7 was the repeating mismatched antigen with the failing first kidney allograft. The newly emerged antibody to HLA-DR7 probably is the consequence of bystander activation of memory response by the COVID-19 vaccination. This case highlights the importance of verifying allo-sensitization history and utilizing multiple assays, including cell-based crossmatch and solid-phase assays with multi-antigens. COVID-19 immunization may deserve special attention when assessing the immunological risk before and after organ transplantation.

KEYWORDS
alloantibody, clinical research/practice, crossmatch, histocompatibility, immunobiology, kidney transplantation/nephrology, kidney transplantation: living donor, panel-reactive antibody (PRA), translational research/science, vaccine

1 | BACKGROUND

Infection and vaccination have been reported to be associated with allo-sensitization either in healthy population or in solid organ transplant recipients.[1-4] The effect of COVID-19 infection and vaccination on antibodies to human leukocyte antigen (HLA) in transplant candidates and recipients is unknown. Herein, we reported a case of positive flow cytometry crossmatch (FCXM) in a renal transplant candidate following a recent COVID-19 vaccination. Interestingly, the donor-specific antibody (DSA) was not

detectable with the sensitive solid-phase single-antigen beads (SAB) assay.

2 | CASE

A 53-year-old male patient with end-stage renal disease secondary to membranoproliferative glomerulonephritis received a kidney transplant from a deceased donor in 1994. Due to recent biopsy-proven advanced interstitial fibrosis and tubular atrophy, his first allograft was failing (serum creatinine = 5.1 mg/dL and estimated glomerular filtration rate = 12 ml/min/1.73 m^2). He was evaluated for preemptive retransplantation from a related living donor (nephew). The patient has been maintained on stable triple immunosuppression of cyclosporine, azathioprine, and prednisone without recent modifications. Recently, there were no significant medical or surgical events, such as hospitalizations or blood product administrations.

The patient's HLA typing is A*02:01, 24:02; B*15:01, 44:02; Bw4, 6; C*03:03, 05:01; DRB1*04:01, 04:03; DRB4*01; DQA1*03; DQB1*03:01, 03:02; DPA1*01:03; and DPB1*04:01. The HLA typing for the living donor candidate is A*01:01, 11:01; B*35:01, 57:01;

FIGURE 1 Results of solid-phase anti-HLA antibody testing. The first serum with the multi-antigen PRA beads (A) and the single-antigen beads (B). The second serum with the multi-antigen PRA beads (C), the single-antigen beads (D), and the Reflex beads (E). The mismatched donor HLA-DR7 is highlighted [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of flow cytometry crossmatch with different donors

	HLA-DR		T-FCXM (MCS)[a]		B-FCXM (MCS)	
			First serum	Second serum	First serum	Second serum
Patient	4		NT	NT	NT	NT
First kidney graft	4	7	NT	NT	NT	NT
Living donor candidate	1	7	10	10	0	**1278**
Third-party donor #1	4	15	13	110	100	126
Third-party donor #2	4	15	0	0	0	0
Third-party donor #3	15	17	0	7	43	40
Third-party donor #4	7		0	23	76	**1432**
Third-party donor #5	7	15	28	20	72	**1212**

Abbreviations: B-FCXM, B cell flow cytometry crossmatch; MSC, median channel shift; NT, not tested; T-FCXM, T cell flow cytometry crossmatch.

[a]The standard three-color flow cytometry crossmatch was performed with pronase-treated lymphocytes. The threshold for positive FCXM is 150 MCS; positive FCXM is highlighted in bold.

C*04:01, 06:02; DRB1*01:01, 07:01; DRB4*01:03N; DQA1*01, 02; DQB1*03:03, 05:01; DPA1*01:03, 02:02; and DPB1*03:01, 04:01. The patient's anti-HLA class I antibodies were negative with both multi-antigen panel-reactive antibody (PRA) beads and SABs (One Lambda). Class II PRA was 20% (Figure 1A); class II antibodies to HLA-DPB1*02:01, 06:01, 09:01, 10:01, 13:01, 17:01, and 19:01 were identified with the SAB assay (Figure 1B). The mean fluorescence intensity (MFI) for these positive beads ranged from 6055 to 16399. The antibody profiles were concordant in SABs and multi-antigen beads and were also consistent with two historical sera collected 5 and 10 months before. These HLA-DP shared an epitope 69E (glutamic acid), which is known to be involved in peptide binding and T cell receptor recognition.[5] Noteworthily, donor-mismatched antigen HLA-DPB1*03:01 has lysine (K) at amino acid position 69 instead and was negative in SAB assay. The initial FCXM with the living donor candidate was negative for both T and B cells (Table 1). These results were concordant with a lack of DSA in the patient's serum.

One hundred and sixty-seven days after the initial FCXM, a final FCXM was performed using a fresh serum sample from the patient (designated as the second serum). Although T cell FCXM was still negative, there was a surprisingly strong positive FCXM on B cells (median channel shift [MCS] = 1278; the positive threshold is 150 MCS). The patient had not received any therapeutical antibodies such as Rituxan, which were known to promote false-positive B cell FCXM. Pronase-treated lymphocytes were routinely used in FCXM to reduce the false positivity on B cells. The antibody profile of the second serum on SAB was similar to the first serum (Figure 1D). Therefore, it appeared that the B-FCXM was positive in the absence of DSA detected with the sensitive SAB assay. However, class II PRA was increased to 37% from 20% in multi-antigen PRA beads on Luminex platform with a new pronounced HLA-DR7 pattern (Figure 1C). With another multi-antigen PRA beads on flow platform (One Lambda), the class II PRA also increased from 14% to 29% (data not shown). This HLA-DR7 pattern in PRA beads was absent in the first serum used in the initial crossmatch (Figure 1A) and two additional historical sera. HLA-DR7 was identified as the mismatched

antigen with the living donor candidate. Thus, the positive B cell FCXM was supported by the presence of HLA-DR7 DSA identified with multi-antigen PRA beads but not with SAB.

Were there any prozone or interference agents that covered the HLA-DR7 antibodies in the SAB assay? In our laboratory, sera are routinely treated with ethylenediaminetetraacetic acid (EDTA) to remove prozone caused by possible complement complexes.[6] To exclude any EDTA-independent prozone effects, we performed additional dilutions of the EDTA-treated sera (1:4 and 1:16) and did not find any new reactivity. In addition, the removal of IgM by heat inactivation (63°C) did not uncover any new antibodies. No DSA was found in the Luminex-based C1q assay (One Lambda) or a SAB assay from an alternative vendor (Immucor). We also tested the postvaccination serum with a new Reflex bead assay (One Lambda), coated with rather native HLA class II single antigens. The profile was similar to the regular SAB, and MFI for the DR7 bead was only 373 (Figure 1E). DSA at such a level usually is considered negative and incapable of causing the strong positive B cell FCXM.

To better characterize the allo-specificity of the positive FCXM we observed, we tested both sera with five third-party surrogate donor cells (Table 1). Three surrogates (#1-3) were negative for HLA-DR7, while two other donors (#4 and #5) expressed HLA-DR7. There were no DSA to HLA-DP for any of the five surrogate donors (Table S1B). With HLA-DR7–negative donors (n = 3), both sera were negative in B cell FCXM. In contrast, when HLA-DR7–positive donors (n = 2) were used, the B cell FCXM was strongly positive (MSC = 1432 and 1212, respectively) with the second serum while negative with the first serum. Thus, the second serum exhibits allo-specificity toward donor HLA-DR7 antigen.

What are the possible sources of allo-sensitization for the HLA-DR7 antibodies? HLA-DR7 was the mismatched antigen from the failing first kidney transplant (HLA typing: A23, 24; B62, 44; Bw4, 6; and DR4, 7). Although HLA-DR7 DSA to the first kidney allograft was never detectable in peripheral blood during the limited

follow-up period, the presence of memory B cell responses to HLA-DR7 is plausible. The patient received a two-dose COVID-19 vaccine (Pfizer-BioNTech) 37-days before collecting the second serum. Thus, the COVID-19 vaccination may have activated the memory response to HLA-DR7. After reviewing the positive FCXM caused by HLA-DR7 DSA, and sensitization history, the transplant clinicians decided not to proceed with this living donor candidate for kidney transplantation. Instead, the patient received a FCXM-negative, low-risk donor through kidney paired donation.

3 | DISCUSSIONS

Recent advances in the solid-phase assay for HLA antibody detection have significantly improved the ability to assess histocompatibility for organ transplantation. Mainly, SAB assay makes the accurate identification of antibody specificity much more accessible than ever. However, there are well-known false-positive reactions on SAB assay, which are usually caused by denatured antigens on beads.[7-10] Anti-HLA antibodies reactive to cryptic epitopes on SAB are often negative when tested with cell-based crossmatch[11,12] and clinically irrelevant.[13] Prozone is the primary source of false negativity in SAB; usually, it can be mitigated with EDTA, dithiothreitol, or serum dilutions.[14,15] Here, we reported a case of false-negative HLA-DR7 antibody in solid-phase SAB assay excluding possible interference from prozone. The HLA-DR7 antibody was clearly identified with cell-based assays such as FCXM. Interestingly, albeit lack of sensitivity, the multi-antigen PRA beads displayed a clear pattern of HLA-DR7 reactivity. The antigens on multi-antigen PRA beads are extracted from EBV-transformed B cells derived from human donors, which are similar to B lymphocyte cells used in FCXM. In contrast, the antigens on SAB are recombinant proteins expressed in cell lines. HLA-DR is an alpha/beta heterodimer that requires a chaperon, class II-associated invariant chain peptide (CLIP) for antigen presentation and structural stability.[16] One hypothesis is that the cell lines producing the single antigens lack CLIP, while the cell lines generating the multi-antigens as well as human B cells used in FCXM have CLIP.[17,18] In a case of a false-negative antibody to HLA-DRB5*01:01 with standard SAB assay, the antibody was detectable when single-antigen cell lines were transfected with CLIP.[17] Similarly, in this case, HLA-DR7 on SAB may have an altered configuration that may not be recognized by the HLA-DR7 antibodies. The manufacturer recently produced new Reflex beads conjugated with HLA class II antigen expressed in a CLIP-positive cell line.[18] The Reflex beads appeared to be more sensitive than regular SAB beads in detecting anti-DR7 antibodies. However, the strength (MFI = 373, Figure 1E) was not comparable to the strong reactivity in multi-antigen beads and strong positive B cell FCXM. Alternatively, the peptides bound to HLA were reported to have an impact on the specificity of anti-HLA antibodies.[19] Possibly, the HLA-DR7 antibody recognized the HLA-DR7/peptide complex. The difference of peptide repertoire in the SAB cell lines vs. PRA bead

cell lines and normal lymphocytes may explain the different reactivities of HLA-DR7 antibodies in our case.

The SAB assay is routinely used in histocompatibility laboratories to determine unacceptable antigens for virtual crossmatch.[20] The pros and cons for virtual vs. cell-based crossmatches were summarized in a recent viewpoint paper.[21] Our case highlighted the importance of applying multiple tests to comprehensively assess histocompatibility and resolve the discrepancies between the solid-phase and cell-based assays. Especially the multi-antigen beads and FCXM with surrogate donors are still helpful tools in histocompatibility laboratories.

COVID-19 vaccine would provide hope for the pandemic while also create challenges for the transplantation community. Much of our knowledge regarding vaccine-induced anti-HLA antibodies was from influenza vaccines. One study reported 11.9%–17.3% of patients have positive anti-HLA antibodies following H1N1 vaccination.[4] However, a meta-analysis[22] and a recent report[23] indicated that the incidences of de novo DSA and rejection after H1N1 vaccination in solid organ transplant recipients were rather low. Several mechanisms have been proposed for the vaccination effects—immune mimic, the adjuvant effect on innate immunity, and bystander activation of quiescent alloreactive memory.[24] The single-stranded mRNAs in COVID-19 vaccines are potent stimulators for Toll-like receptors on B cells,[25] promoting a robust bystander activation of preexisting, HLA-DR7–specific memory B cells. Several approaches, such as HLA tetramers[26] or B cell ELISPOT,[27,28] were reported to detect HLA-specific memory B cells in peripheral blood of sensitized patients. These assays potentially predict the recall of the humoral memory response to HLA, complementary to the regular serological antibody tests. However, the analytic validity and clinical utility for these B cell assays still need to be determined for their routine clinical usages. Thus, when evaluating organ transplant candidates, previous sensitization history shall be confirmed, and immunizations shall be considered sensitization events. This is specifically relevant for patients receiving organ transplants shortly after vaccination, such as patients waiting for thoracic organs. For previously sensitized transplant candidates, the risk for activating memory responses and detecting new anti-HLA antibodies after vaccination would be high. An additional HLA antibody test 1 month after COVID-19 vaccination might be necessary to ensure that transplantation proceeds safely without the risk of new DSA. While advocating caution, we acknowledge the frequency and clinical relevance of this type of events are unclear. Thus, this case does not discourage vaccination of COVID-19 or any other viruses in transplantation patients. More studies will be needed to determine the impact on anti-HLA antibodies following COVID-19 vaccination in transplant candidates or recipients.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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SUPPORTING INFORMATION

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