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BRIEF COMMUNICATION

HLA Immune Response Genetics WILEY

SARS Cov-2 vaccination induces de novo donor-specific HLA antibodies in a renal transplant patient on waiting list: A case report

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The ability of COVID-19 vaccination to induce anti-HLA antibodies (Abs) formation in renal transplant candidates is not well studied. A 42-year-old man on a renal transplant waitlist, with no sensitization history, was tested for DSA before and after COVID-19 vaccination. Patient has consistently tested negative for COVID-19 virus. Eighteen days after receiving first dose of mRNA-based vaccine, flow cytometry crossmatch (FCXM) was strongly positive with de novo donor-specific Ab (dnDSA) against B57 and de novo non-DSA against B58. Before vaccination, preliminary FCXM was negative with no anti-HLA Abs. This event prompted the transplant team to cancel the surgery. COVID-19 vaccination could be associated with anti-HLA Abs formation in renal patients on waitlists that could affect future transplantability.

KEYWORDS
donor-specific HLA antibodies, renal transplant patient, SARS Cov-2 vaccination

1 | INTRODUCTION

The ongoing SARS-CoV-2 (COVID-19) pandemic has changed the infectious and vaccination status of patients on solid organ waiting lists. Recently, multiple studies have been published examining the response of COVID-19 mRNA-based vaccines in solid organ transplant recipients.[1–6] While COVID-19 vaccination is considered the best medical solution for controlling the pandemic, the potential of vaccination to induce the formation of anti-HLA antibodies (anti-HLA Abs), especially de novo donor-specific Ab (dnDSA) that can halt solid organs transplantation, is still under investigation.[3,7,8] While the risks of COVID-19 vaccines in respect to anti-HLA Abs induction is still unclear, it is documented that some vaccines, such as seasonal influenza and pneumococcal vaccines, can be associated with re-stimulating or activating memory B cells that have been previously formed after historical sensitizing events leading to induction of anti-HLA Abs production in solid-organ transplant recipients.[9–11] A recent report also showed a possible reactivation of Abs against repeat mismatched class II DR antigen post COVID-19 vaccination.[12]

Any change in the anti-HLA Abs pool in potential solid organ transplant recipients can dramatically affect the flow cytometric cross matching (FCXM) and the success of transplants as even low incidences of anti-HLA Abs are shown to induce solid organs rejection.[11] Few publications have documented the formation of anti-HLA Abs and DSA in some solid-organ transplant recipients after COVID-19 or Pseudomonas aeruginosa infections,[13–16] that may cause chronic active Ab-mediated rejection (AMR).[15] In this article, we present the first case report of a patient on a renal transplant waiting list with no history of sensitization, developing dnDSA and de novo non-DSA following COVID-19 vaccination.

2 | CASE PRESENTATION

A 42-year-old man with B positive blood group, has been on a kidney transplant waiting list for several years, has consistently tested negative for COVID-19 virus and been routinely worked up for a renal transplant (Table 1). The patient has received two doses of a COVID-19 mRNA-based BNT162b2 (Pfizer-BioNTech) vaccine. He has no previous history of sensitization or sensitizing events such as previous transplants or blood product transfusions. All of the patient's molecular testing results for COVID-19 infection, using RNA-NAT, were negative before preliminary and final FCXM, but no serological testing for COVID-19 infection, using anti-nucleocapsid Abs, was performed. To rule out autoimmunity, the patient was screened for many autoantibodies including anti-nuclear Abs (ANA), phospholipase A2 receptor Abs (PLA2R), anti-neutrophil cytoplasmic Abs (ANCA), myeloperoxidase Abs (MPO) and proteinase 3 Abs (PR3). All autoantibody screening results, before COVID-19 vaccination, were negative. Moreover, the patient's history of vaccination was reviewed for influenza (FLU); Pneumococcal conjugate (PNEUMO-C); and Pneumococcal polysaccharide 23 (PNEUMO-P). We performed epitope analysis using HLAMatchmaker to determine if shared eplet could account for the HLA-B57/B58 pattern of reactivity, based on the donor and recipient HLA profile.

3 | RESULTS

Before COVID-19 vaccination, as a part of routine preliminary testing blood workup for an intended donor, HLA Ab screening was performed using HLA class I and II antigens loaded to microbeads and (LABScreen® Mixed Antigen Beads, One Lambda, Canoga Park, CA) tested by Luminex screening approach. Reactivity against single antigens was further tested (LABScreen® Single Antigen Beads [SAB], One Lambda) for HLA class I (Figure 1A) and class II (Figure 1B), at which both showed no anti-HLA Abs before COVID-19 vaccination with a calculated panel reactive antibodies (cPRA) of 0%. In our center, we assign as positive result to any reactivity to HLA antigens exceeding a normalized mean fluorescence intensity (MFI) value of 1000 in the SAB assay. HLA Cw, DQ, and DP are assigned based on higher MFI cutoff given their lower expression. Development of anti-HLA Abs was defined as any de novo induction in reactions after vaccination as compared with baseline. As per our protocol, all neat sera were treated with ethylenediaminetetraacetic acid (EDTA) to rule out any prozone effect or interference, caused by possible complement complexes. To exclude any EDTA-independent prozone effects, we performed additional dilution of the EDTA-treated sera (1:10) and did not find any new reactivity.

Eighteen days after the patient received his first dose of COVID-19 vaccination, dnDSA against B57 (MFI 12,211) and de novo non-DSA against B58 (MFI 10,920) were detected (Figure 1C) in final testing for transplantation with cPRA of 9% (Figure 1D). These de novo Abs were not in patient's tested serum five months ago, at the time of preliminary FCXM and SAB testing, also was detected only one day after second COVID-19 vaccination. A dilution of neat serum (1:10) was performed to test for any hidden reactivity before vaccination and showed the same negative results with MFI values of less than 80 (Figure 1E). Preliminary FCXM, before vaccination, showed negative results in both autologous and allogenic (Figure 2A) T cell (MCS 3SD shift −25, cutoff 66) and B cell (MCS 3SD shift −22, cutoff 97) crossmatches.

TABLE 1 Patient and donor characteristics and FCXM results for donor and surrogate cells

					FCXM results	
	Age (years)	Sex	Blood type	HLA typing	T cells	B cells
Patient	42	Male	B+	A24,30;B13,76;Bw4,6;Cw6,9; DR7,12;DRw52,53;DQ2,7;DP3,17	Negative	Negative
Donor	46	Female	B−	A26;B38,***57***;Bw4;Cw6,12; DR4,7;DRw53;DQ8,9;DP0201,10	Positive	Positive
Surrogate cells #1	NA	NA	NA	A11,32;B27,35;Bw4,6;Cw1,4; DR1,14;DRw52;DQ5;DP3,16	Negative	Negative
Surrogate cells #2	NA	NA	NA	A23,29;B44;Cw4,16; DR7,15;DRw51,53;DQ2,6	Negative	Negative
Surrogate cells #3	NA	NA	NA	A1,29;B55,***57***;Cw1,6; DR13;DRw52;DQ6;DP3,0401	Positive	Positive

Note: HLA class I antigens against which the recipient has developed dnDSA have been highlighted as bold and italicized.
Abbreviations: FCXM: flow cytometry crossmatching; NA: not applicable.

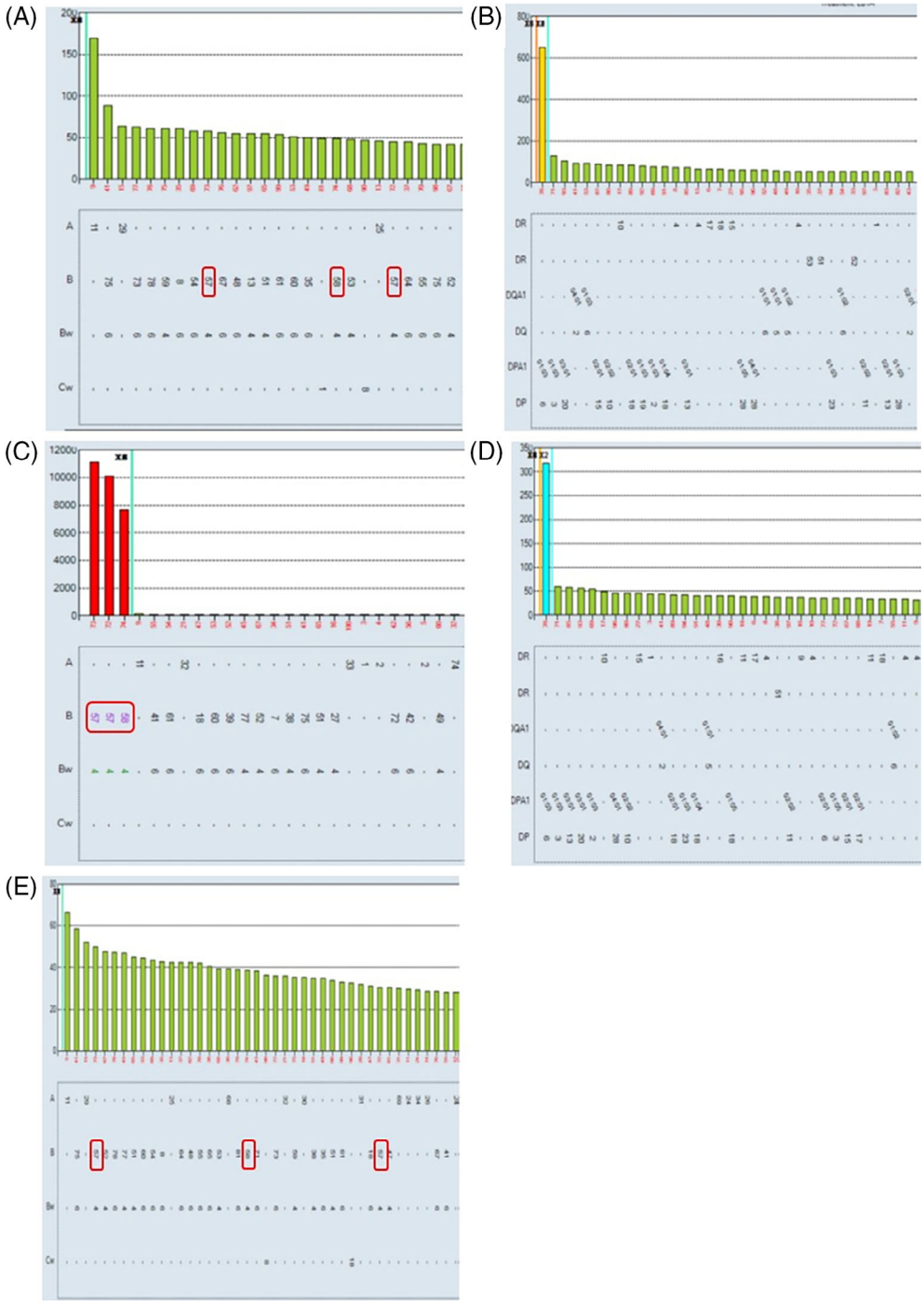

FIGURE 1 HLA antibodies analysis by LABScreen® Single Antigen Bead (SAB). Luminex results before COVID-19 vaccination for class I (A) and class II (B) compared with after vaccination for class I (C) and class II (D), and 1:10 diluted sample before vaccination (E)

In the final FCXM performed after vaccination, T cells (MCS 3SD shift −18) and B cells (MCS 3SD shift −40) were both negative in autologous setting, but strongly positive in T cells (MCS 3SD shift 185, cutoff 66) and B cells (MCS 3SD shift 149, cutoff 97), as shown in Figure 2B. Owing to the presence of dnDSA and strongly positive final crossmatching, the scheduled transplant was canceled. Both dnDSA and positive crossmatching were confirmed by repeating the SAB and FCXM testing. This de novo Ab formation was triggered by first dose of the vaccine as patient received the second vaccination dose only one day before testing for final FCXM and Ab screening and SAB. Lately, patient received a third vaccine dose.

For better characterization of the patient's allo-specific dnDSA of the positive FCXM, post-vaccination serum was tested with different surrogate cells (Table 1). First and second surrogate cells were not carrying neither HLA-B57 nor HLA-B58, and gave negative FCXM results for both T and B cells. On the other hand, the third

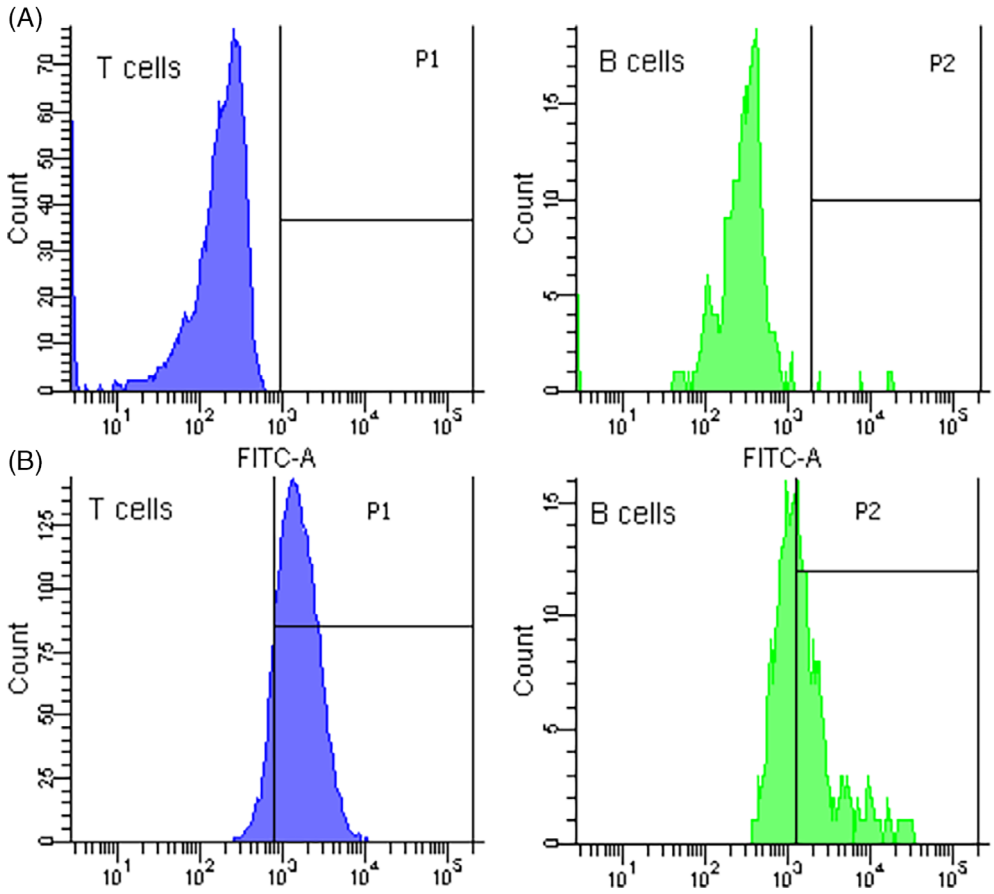

FIGURE 2 Flow cytometry crossmatching (FCXM). (A) Results of pre-COVID-19 vaccination preliminary FCXM compared with (B) post-COVID-19 vaccination final FCXM. Pre- and post-vaccine T and B cell autologous FCXM gave negative results (data not shown). Fractions of allo-reactive T cells (P1) and B cells (P2) were plotted

surrogate cells carrying only HLA-B57, gave positive FCXM results with both T and B cells obtained confirming the allo-specificity against HLA-B57.

Finally, to check if the formation of this Abs was temporal, five months after vaccination both Abs against HLA-B57 and HLA-B58 were tested by SAB (Figure 3). The reactivity to HLA-B57 and HLA-B58 diminished over time but was still detectable with MFI scores of 3433 and 2352, respectively, which were still detectable by FCXM. When FCXM was repeated, it was found to still be positive but with a reduction in MCS for both T cells (MCS 3SD shift 121) and B cells (MCS 3SD shift 102).

Epitope analysis, using HLAMatchmaker, revealed three common eplets between HLA-B57 and HLA-B58, namely 62GE, 69AA, and 71SA. One or more of these three eplets could be accounted for the HLA-B57/B58 pattern of reactivity, based on the donor and recipient HLA profile.

At the time of final editing of this manuscript, the patient successfully matched in a kidney paired exchange (KPD) program with a donor who lacks HLA-B57 and HLA-B58 and both negative T- and B-cell FCXM. If this KPD offer comes to term with a successful surgery, the patient would have spent 5 additional months on dialysis because of this suspected COVID-19 vaccine induced DSA.

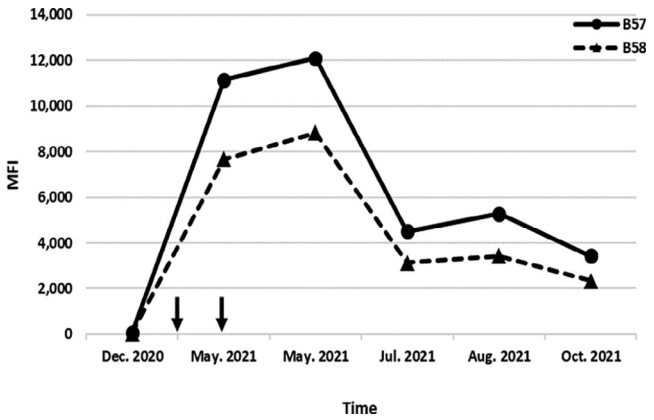

FIGURE 3 Mean fluorescence intensity (MFI) monitoring for anti-HLA Abs before and after vaccination. Arrows indicate time of COVID-19 vaccination doses

This study was approved by the University of Calgary Institutional Review Board (REB13-0682).

4 | DISCUSSION

Given the extent of the COVID-19 pandemic and vaccination trends, solid organ transplant recipients and patients

on waiting list should be specifically considered for unfavorable side effects of COVID-19 infection and vaccination. It has been documented that COVID-19 infection could induce anti-HLA Abs development in solid organ transplanted recipients.[13,15] This effect is most likely because of reducing the dose of immunosuppressant medications taken by patients to help overcome COVID-19 infection. In such cases, patients' immune system becomes more competent to mount an immune response to the transplanted organ. It is a challenge in the COVID-19 era to balance dosages of immunosuppressing medications to allow proper immune response to the invading virus while keeping transplanted organs tolerable to the transplanted patient's immune system.

Vaccination is good way to protect transplanted patients from infection, but this is counter balanced by the fact that some vaccines are known to induce patients' immune system to develop anti-HLA Abs and even DSA. Many published works describe the ability of influenza virus immunization to induce dnDSA reaching incidences of 1.85% at 21–94 days post-transplant.[11] While we have also previously reported a significant impact of influenza virus vaccination on anti-HLA Ab production, that was detected in only 1.4% of solid organ recipients but with no clinical consequences.[16] On the other hand, in a meta-analysis of vaccination side effects in solid-organ transplants, it was found that the incidence of rejection was 2.1% at 0.7 to 6 months after transplantation in vaccinated patients.[11] Other vaccines such as Pneumococcal vaccines have also been shown to change the pool of anti-HLA Abs in kidney transplant recipients.[10] Lindemann et al showed that Pneumococcal vaccination can significantly increase the HLA Abs at 1 to 12 months after vaccination in female, but not male, renal transplanted patients.[10] In our case report, patient's previous influenza and Pneumococcal vaccinations as a causative agent for the formation of these de novo anti-HLA Abs were excluded because of the absence of anti-HLA Abs and negative FCXM in patient's serum sample before COVID-19 vaccination.

Little is known about the effect of COVID-19 vaccination on the ability of transplanted patients to form anti-HLA Abs. The limited available literature has identified the generation of dnDSA in renal transplanted patients after COVID-19 infection even with only increasing steroids and unchanged calcineurin inhibitors (CNI) and mycophenolate mofetil (MMF/mTORi).[8] A small sample size study, documented that COVID-19 infected and recovered candidates on waiting lists can proceed with solid transplantation with no impact on anti-HLA Abs profile in a series of waitlisted renal transplant patients.[7] To the best of our knowledge and compared with the possibility of reactivation of Abs against repeat mismatched class II DR antigen post COVID-19 vaccination,[12] this is the first report describing the effect of COVID-19 vaccination on inducing the development of de novo anti-HLA Abs. Unfortunately, in this novel case, it was dnDSA that stopped a planned living donor renal graft.

Induction of anti-HLA Abs after vaccination may be attributable to many factors including T- and B-cell immune responses to vaccine antigens that directly may cross-react with alloantigens such as is thought to occur in some viral infections.[17] Additionally, innate immune responses to vaccination, including cytokine release, may stimulate previously quiescent alloreactive memory responses.[17] Another mechanistic possibility is the effect of adjuvants used in some vaccines that may lead to non-specific immunostimulating effects.[18] Many reports about the increased rates of rejection and DSA formation after the use of vaccines containing the ASO3 adjuvant have been produced since it was first used in the 2009 H1N1 influenza pandemic.[18] In this case, the patient received many influenza and Pneumococcal vaccines in years previous to COVID-19 vaccination. This led to a hypothesis that one dose of COVID-19 vaccination may have activated the immune system because of a putative adjuvant effect or cross-reaction with other autoantigens generating anti-HLA Abs, that, in this case, were dnDSA in addition to de novo non-DSA HLA Abs belonging to the same parent antigen HLA-B17 (HLA-B57, HLA-B58). Taking into consideration that there were reductions in HLA-B17 broad antigen titer and in the strength of FCXM of both T and B cells three months after vaccination, it may be possible that the formation of anti-HLA Abs can be temporal.

Considering that both HLA-B57 and HLA-B58 have 62GE, 69AA, and 71SA as common eplets, so the de novo Abs pattern of reactivity, based on the donor and recipient HLA profile, seem to be eplet related. Especially that these three eplets are known to be immunogenic and able to induce anti-HLA Abs.

In light of previously documented effects of other vaccines and COVID-19 infection on Ab formation, the case of this patient, with no previous history of sensitization, no COVID-19 infection, and no autoimmunity, should be considered as a potential limitation of vaccination for some patients on renal transplant waiting lists. Caution should be taken with renal patients waiting for solid organ transplant and screening for development of dnDSA after single or double doses of COVID-19 vaccines may be required if this hypothesis is confirmed. Our laboratory is currently conducting a systematic review of more patients who have received a variety of vaccines to assess the rate of occurrence of de novo-HLA Abs post-COVID-19 vaccines in renal transplant patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ahmad Abu-Khader, research performing, data analysis and interpretation, drafting and revising the manuscript. Wenjie Wang, transplant nephrologist providing patient samples, clinical outcomes and sensitization history. Iwona Galaszkiewicz and Faisal Khan, reviewing and reporting histocompatibility work. Meriam Berka, critical review of the manuscript. Noureddine Berka, research concept and design, revising the manuscript, and final approval for journal submission.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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