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HLA Sensitization in the Era of COVID-19: Single-Center Experience

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ABSTRACT

It is well known that several viral infections are capable of triggering the formation of HLA antibodies; however, an association between SARS-CoV-2 and the development of anti-HLA antibodies is not yet confirmed. In this study, we compared the prevalence of HLA antibody before and after COVID-19 infection in a cohort of 3 groups included 58 healthy nonsensitized employees (HNEs), 130 kidney transplant recipients (KTRs), and 62 kidney transplant candidates. There were no significant changes observed in HLA class I antibodies in any of the groups, but evaluation of antibodies to HLA class II revealed a significant change in the KTR group (P = .0184) after acquiring COVID-19 infection and in the HNE group (P = .0043) when compared to the reported prevalence in a similar population. Although we observed the emergence of convalescent de novo donor-specific antibodies in 2 patients, we did not encounter any rejection episodes in the KTR group. Finally, the results of flow cytometry crossmatch in the HNE group were not consistent with the state of antibodies. In conclusion, COVID-19 infection has the potential to produce class II antibodies but with little effect on preexisting sensitization. These antibodies are likely to be transient and not necessarily causing positive crossmatch with the corresponding antigens at the proper mean fluorescent intensity and therefore should not affect access to transplantation. There is a need for further evaluation to ascertain the genuineness of these antibodies and their exact effect on transplant readiness and outcomes.

HLA antibodies have been associated with all forms of antibody-mediated rejection and graft loss [1]. Although HLA antibodies are typically produced after transplantation, pregnancies, and blood product transfusions, some viral infections are capable of inducing HLA antibody production or enhancing existing antibodies [2]. Viral antigens can trigger an immune response to allogeneic HLA antigens via different immunologic mechanisms including heterologous immunity (allo-HLA cross-reactivity with virus-specific T and B cells) and bystander activation of quiescent alloreactive memory cells created by general inflammation associated with the viral infection [2]. Among the viruses that commonly induce HLA antibody production are cytomegalovirus, influenza, varicella zoster, and herpes viruses [2−5]. The role of SARS-CoV-2 exposure in triggering HLA sensitization is still not clear, and the little available data include only a few anecdotal case reports or short series with some controversial results. Although Juskewitch et al initially showed elevated rate of HLA antibodies in male COVID-19 convalescent plasma [6], a second report by the same group on a larger cohort reported no significant association [7]. Another 2 brief reports by Gandolfini et al and Roll et al, respectively on 7 transplant recipients and 18 patients awaiting transplantation who contracted COVID-19, showed no developed HLA antibodies or an increase in the preexisting

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HLA antibodies in highly sensitized patients despite the presence of detectable anti-SARS-CoV-2 antibodies [8,9].

With the implementation of COVID-19 vaccination policies worldwide, further HLA allo-sensitization is possible because vaccination has the potential to induce HLA sensitization in a similar way to infection. The innate immune responses to vaccination include cytokine release capable of stimulating quiescent alloreactive memory responses in addition to the possible immunostimulating effects caused by the adjuvants used in some vaccines [10−13]. Indeed, the single-stranded mRNAs in COVID-19 vaccines were recently found to be potent stimulators for Toll-like receptors on B cells, resulting in release of multiple inflammatory mediators and activation of preexisting HLA alloreactive memory B cells [14]. Moreover, the 2 reported cases that described positive crossmatches due to emergence of de novo HLA antibodies were both preceded by SARS-CoV-2 vaccination, although single antigen bead (SAB) assay identified donor-specific antibodies (DSAs) in only 1 of them [15,16]. Therefore, more studies are needed to confirm the association between SARS-CoV-2 exposure and HLA sensitization and to verify its relevance. The potential role of vaccinations in reinforcing a previous allo-sensitization due to COVID-19 natural infection needs to be considered as well during interpretations of HLA antibody results in view of mass vaccinations.

Our recent observation (data not shown) of many false positive flow crossmatch results in nonsensitized patients during the COVID-19 pandemic in the context of high-scale vaccine programs in our region encouraged us to look into the association between SARS-CoV-2 exposures and HLA antibody productions. In this study, we investigated the association between SARS-CoV-2 exposures and HLA antibody induction and their relevance in a cohort of 3 groups: (1) healthy nonsensitized employees (HNEs), (2) kidney transplant recipients (KTRs) after transplantation, and (3) kidney transplant candidates (KTCs) awaiting transplantation. The study was approved by the institutional review board at King Fahad Specialist Hospital −Dammam.

MATERIALS AND METHODS

Cohorts

This study includes evaluation of HLA antibody profiles before and after COVID-19 infection in the KTR and KTC groups and comparing the prevalence of HLA antibodies with the reported prevalence in a similar population in the HNE group. All subjects in our study had mild to moderate reverse transcriptase polymerase chain reaction−confirmed COVID-19 infection (as defined by the Centers for Disease Control and Prevention [17]) and received at least 1 dose of SARS-CoV-2 vaccine. Ninety-percent of the population also screened positive for SARS-CoV-2 immunoglobulin (Ig) G with or without IgM antibodies.

The first group included sera from 58 HNEs collected within 3 to 6 weeks of COVID-19 infections. All received one to 2 doses of messenger RNA−based SARS-CoV-2 vaccine. The second group comprised 130 KTRs who acquired COVID-19 infection within the previous 4 months referred for routine follow-up. The last group included 62 KTCs and were mixtures of deceased donor waitlisted and living donor candidates who acquired COVID-19 infection within the previous 4 months. Patient characteristics are presented in Table 1. From our database, we retrospectively compared HLA antibody profiles for the second and third groups (KTR and KTC) before and after infection to investigate emergence of de novo antibodies. We also checked for possible sensitizing events or rejection episodes in KTRs.

Table 1. Demographic Characteristics of the 3 Cohorts Included in the Study

Cohorts	Age (y): Mean ± SEM	Sex: M/F
Healthy nonsensitized employees (n = 58)	36.3 ± 3.4	54/4*
Kidney transplanted recipients (n = 130)	19.4 ± 1.9	90/40
Kidney transplant candidates (n = 62)	23.3 ± 2.8	36/26

* The 4 women were single nulliparous women.SEM, standard error of the mean.

Anti-HLA Antibody Testing

HLA antibody screening and SAB assays were performed using One Lambda kits utilizing Luminex platform (One Lambda Inc., Canoga Park, Calif, United States) as previously described [18]. A cutoff point of 1000 mean fluorescent intensity (MFI) was used to report positive specificities. We reported a change in antibody profile if a significant increase of existing specificity occurred (eg, change of weak antibody to moderate) or new specificities emerged according to established cutoff points.

Flowcytometry Crossmatch

Sera were crossmatched with surrogate cells using standard 3-color Flowcytometry crossmatch (FCXM) on an FACS Canto II (BD Biosciences, San Jose, California, USA) as previously described [19]. Cutoffs of >66 mean channel shift (MSC) and >100 MSC were considered positive for T and B cells, respectively. An FCXM result was considered concordant when positive in the presence of DSA >3000 MFI or negative in the absence of DSA or when the DSA was <3000 MFI.

SARS-CoV-2 Detection

Molecular detection of SARS-CoV-2 was performed using reverse transcriptase polymerase chain reaction assay (Xp-sars-cov2-10 KIT, CEPHEID, Sunnyvale, California, USA) and SARS-CoV-2 antibody detected by SARS-CoV-2 S1-S2 IgG/IgM (DiaSorin, Saluggia, Italy) on the Liaison platform.

Statistical Analysis

The chi-square test of significance was used to calculate the P value considering a significance level of .05.

RESULTS

Group 1 (HNE)

HNEs demonstrated a prevalence of 55% and 28% for class I and II antibodies, respectively. The most frequent class I specificities detected included Cw17, B76, and B45 commonly observed in nonsensitized individuals and often considered false HLA specificities. However, 10% of this group revealed a pattern of specificities consistent with A2 cross-reacting epitope

Table 2. Prevalence of HLA Antibodies After Exposure to COVID-19

	Positive, *n* (%)	Frequent Specificities	*P* Value
In healthy nonsensitized employees			
Class I antibodies	32 (55)	Cw17, B76, B45, A2	.1507
Class II antibodies	16 (28)	DQ5, DQ6	.0043*
In kidney transplant recipients			
Class I antibodies	56 (43)	54 (42)	.8119
Class II antibodies	23 (18)	38 (29)†	.0184*
In kidney transplant candidates			
Class I antibodies	23 (38)	32 (52)	.1184
Class II antibodies	22 (35)	16 (26)	.2832

* Significant at $P < .05$.
† No evidence of rejection.

group (CREG) including A2, A68, A69, B57, and B58. The frequently observed specificities for class II included DQ5 and DQ6 (Table 2). To assess the genuineness of these specificities, we performed FCXM utilizing random 25 sera and surrogate cells. Out of 5 FCXMs that were expected to be positive, only 2 produced concordant results and 20 negative sera gave false positive results within the context of negative auto-cross-matches.

Group 2 (KTRs)

There was no change in class I antibodies (42% compared to 43%, $P = .8119$) but a significant change observed in class II (18% compared to 29%, $P = .0184$) when comparing pre-infection with postinfection prevalence (Table 2). The de novo antibodies presented a scattered pattern, with each specificity encountered only once or twice except for DR7, which appeared 3 times. Two patients showed emergence of class II DSAs after infection of 7000 and 3000 MFI that was not associated with development of rejection.

Group 3 (KTCs)

Overall, there was no significant change in antibody prevalence for both classes: 52% compared to 38% ($P = .1184$) for class I and 35% compared to 26% ($P = .2832$) for class II in pre-infection and postinfection sera, respectively. Twenty-nine percent of this cohort showed a change in their antibody profile in the form of postinfection de novo specificities for class I, class II, or both. The new specificities were scattered with no clear pattern observed.

DISCUSSION

Our knowledge on SARS-CoV-2 is still growing; however, we and others have reported that the majority of COVID-19-infected transplant recipients mount good humoral responses to SARS-CoV-2 despite being on triple immunotherapy, indicating that an intact humoral immune response is capable of producing cross-reacting antibodies [20,21].

The prevalence of HLA antibodies post-COVID-19 in our study differed among the 3 groups and showed a significant emergence of class II HLA antibodies in HNEs and KTRs but not in KTCs. Because all candidates in our study were young and had mild to moderate infections, the effect of severity of infection on variation in HLA immune response might be or might not be important to explain the variation in prevalence and could be related to other factors.

Although we did not have baseline HLA antibody profiles in the HNE group, when compared to the reported prevalence of 11% in a similar nonsensitized population [22], the finding of a prevalence of 28% of class II HLA antibodies was significant ($P = .0043$). Moreover, the reported A2 CREG specificities in our study was different from the reported specificities of natural antibodies (usually directed against infrequent HLA antigens in the general population such as B*76, C*17). Both of these findings suggest a potential role for COVID-19 infection to induce class II HLA immunization possibly through activation of anamnestic response rather than de novo synthesis, a mechanism favored by many authors [2] because these antigens are abundant and ready to trigger immunization. The inflammatory responses associated with COVID-19 infection in this group might had activated existing quiescent memory B cells, resulting in the presence of such specificities. Our finding is also consistent with the reported case of emergence of HLA-B57, B58 (part of the A2 CREG) after receiving the first dose of SARS-CoV2 vaccine [15], which may suggest some sort of molecular mimicry between A2 CREG epitopes and SARS-CoV-2 proteins and might deserve further investigation. Lastly, the high prevalence of HLA antibodies in HNEs can be explained by the boosting effect of natural infection on existing post-SARS-CoV-2 vaccination-specific immunity. This is because 3 dose vaccines are mandatory for all employees in our hospital. Cordero et al found a significant increase in the percentage of patients positive for anti-HLA class I after a second immunization dose compared with 1 dose of vaccination (14.6% vs 3.8%, $P = 0.003$) although no subjects developed significant DSA or rejection episodes [10].

The significant emergence of class II HLA antibodies post COVID-19 infection was also persistence in the KTR group, although the specificities presented as sporadic de novo antibodies. This may be related to the established sensitizing event in the latter group compared to others in the form of transplantation, with many already presenting with pre-infection HLA-

antibodies. It is likely that the infection-related inflammatory mediators broadly activated existing B-memory cells in KTRs and manifested as emergence of de novo HLA class II antibodies or enhanced existing ones in some patients. Looking into the likelihood of adverse effects of convalescent HLA antibodies on posttransplant outcomes, we assessed the development of DSA postinfection and any associated adverse graft outcome. Only 2 patients demonstrated postinfection de novo DSAs with no rejection episodes. Although our observation reveals no adverse effects, the absolute safety of HLA sensitization on transplant outcomes post COVID-19 infection cannot be inferred based on 2 cases. Other possible explanations include that antibodies were not at the proper level to cause rejection or the antibodies could be directed against cryptic epitopes that are exposed on the denatured SAB antigens. On the other hand, they could have caused subclinical rejection that was not tested. Altogether, additional data and studies are required to arrive at a solid conclusion on the adverse effect of COVID-convalescent DSAs on graft outcome.

Proinflammatory mediators released during COVID-19 infection are known to cause a significant increase in breadth and strength of HLA-specific antibodies [23,24]. However, a masking effect of immunosuppressive therapy in KTRs could explain the lower prevalence of HLA antibodies compared to HNEs, who were healthy with no medication effects. In fact, the 2 reported cases of antibody-mediated rejection were due to emergence of DSA post COVID-19 infection, both were preceded by immunosuppressive medication reduction [25,26], which is of special concern in view of the common practice of reducing immunosuppressive medications in transplant recipients in most centers for COVID-19-infected transplant recipients.

The KTC group comprised mixtures of patients with chronic kidney disease of variable duration awaiting transplantation. The nonsignificant allo-sensitization in this group could be attributed to the general poor health status of these patients compared to healthy employees who were capable of mounting strong allo-immune responses, in addition to lack of vaccination in some, because vaccination is only mandatory for admitted patients in our institution. Another contributing factor might be related to declining immune responses due to longer time intervals between infection and serum collections in KTC and KTR groups (1-5 months) compared to HNEs (2-6 weeks). We have shown previously that the durability of SARS-CoV-2 antibodies declined after the fourth month of infection [27]; therefore, a similar mechanism could have operated for the durability of HLA antibodies. Interestingly, follow-up of 5 employees who developed high MFI antibodies revealed disappearance of antibodies by 1 year postinfection, indicating a transient nature for this allo-immunization.

In a separate observation, we demonstrated during transplant workups for transplant candidates (data in the publication process) that many COVID-19-infected patients demonstrated positive crossmatches in the absence of or weak DSA status, which might be due to nonspecific reaction related to immune complexes generated during infection and present in their sera. The finding of an insignificant association between crossmatch results and the HLA antibody status in this study suggests that at least the new antibodies were unlikely to be genuine and possibly directed against denatured antigens. However, such a possibility cannot be confirmed, because only a few sera were crossmatched with corresponding antigen-carrying cells. The effect of HLA sensitization on transplant outcomes cannot also be exactly inferred from this study because only a few KTRs demonstrated de novo DSAs with no rejection episode encountered during the study period.

Our preliminary study showed that COVID-19 infection has the potential to induce HLA antibody production, especially class II; however, these antibodies are likely to be transient and not necessarily cause a positive crossmatch with the corresponding antigens at the proper MFI. Therefore, these should not affect access to transplantation yet need to be evaluated further by alternative laboratory methods. Furthermore, this observation of a possible relationship between COVID-19 and HLA class II antibody stimulation deserves further investigation into the effect of severity of COVID-19 infection on the propensity toward anti-HLA antibody production, which could be a future direction for study.

CONCLUSION

COVID-19 infection and/or vaccination may deserve special attention when interpreting HLA antibodies to assess immunologic risk before denying transplantation to KTCs and careful monitoring of DSAs on large-scale COVID-infected KTR cohorts are needed to confirm their effect on transplant outcomes.

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