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ORIGINAL ARTICLE

Influenza vaccination and humoral alloimmunity in solid organ transplant recipients

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The authors have no conflicts of interest.

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Summary

Annual influenza vaccination is recommended in solid organ transplant (SOT) recipients. However, concerns have been raised about the impact of vaccination on antigraft alloimmunity. We evaluated the humoral alloimmune responses to influenza vaccination in a cohort of SOT recipients between October 2008 and December 2011. Anti-HLA antibodies were measured before and 4–8 weeks after influenza vaccination using a solid-phase assay. Overall, 169 SOT recipients were included (kidney = 136, lung = 26, liver = 3, and combined = 4). Five (2.9%) of 169 patients developed *de novo* anti-HLA antibodies after vaccination, including one patient who developed donor-specific antibodies (DSA) 8 months after vaccination. In patients with pre-existing anti-HLA antibodies, median MFI was not significantly different before and after vaccination ($P = 0.73$ for class I and $P = 0.20$ for class II anti-HLA antibodies) and no development of *de novo* DSA was observed. Five episodes of rejection (2.9%) were observed within 12 months after vaccination, and only one patient had *de novo* anti-HLA antibodies. The incidence of development of anti-HLA antibodies after influenza vaccination in our cohort of SOT recipients was very low. Our findings indicate that influenza vaccination is safe and does not trigger humoral alloimmune responses in SOT recipients.

Introduction

Influenza infection is a cause of significant morbidity in solid organ transplant (SOT) recipients [1,2], and it is associated with an increased risk for viral and bacterial pneumonia, allograft dysfunction, admission to the ICU, and even mortality [2]. Therefore, prevention of influenza is of major importance in this population, with annual administration of influenza vaccine being the main preventive strategy [3]. Despite variations in immunogenicity and efficacy of influenza vaccine after transplantation, vaccination remains strongly recommended in SOT recipients, because of significant reduction in influenza-associated morbidity observed with the use of the vaccine [3–6].

Influenza infection has historically been associated with allograft rejection. Evidence from animal models and human studies suggests that antigens derived from infectious pathogens may cross-react with alloantigens, leading to stimulation of alloimmunity, a phenomenon named heterologous immunity [7]. This observation raised concerns that influenza vaccines, containing inactivated viral antigens, could also influence alloimmune responses [8]. Anecdotal reports of acute rejections following immunizations suggested a potential relationship between influenza vaccination and rejection [9]. However, larger studies could not show an increased risk of acute rejection after influenza vaccination in kidney transplant recipients [10–12]. Of note, few studies have investigated the effect of influenza vaccination on anti-HLA antibody production, with conflicting results [11–13].

The aim of our study was to assess the safety of influenza vaccine by measuring the titers of anti-HLA antibodies before and after vaccination and determining the incidence of rejection episodes occurring within 12 months after vaccination in a large cohort of SOT recipients.

Methods

Patient population

For the present study, we included patients that participated in three influenza vaccine studies performed at our institution from 2008 to 2010 [14–16]. In the first study, lung transplant recipients were randomized to receive either intramuscular or intradermal influenza vaccine [14]. In the second study, kidney and liver transplant recipients received two doses of the adjuvanted H1N1/09 influenza vaccine [15]. The third study evaluated the influence of induction therapy on the immunogenicity of influenza vaccine in kidney transplant recipients [16]. All patients were followed at the outpatient clinic of the Transplantation Center of the University Hospital of Lausanne (CHUV) in Switzerland. Determination of anti-HLA antibodies was performed using stored frozen sera. Inclusion and exclusion criteria were similar in all three studies. Basically, inclusion criteria were age ≥18 years, ≥3 months after transplantation, and no episode of acute rejection over the previous month. Exclusion criteria were pregnancy, egg allergy, and previous adverse events related to receipt of influenza vaccine. All subjects provided written informed consent. Additionally, in 2011, kidney transplant recipients followed at the outpatient clinic of the transplantation center and having received the influenza vaccine at our institution were routinely screened for the development of anti-HLA antibodies after a median time of 8 weeks after vaccination. The median interval between measurement of prevaccination anti-HLA antibodies and vaccination was 1 day (IQR 0–65 days).

Immunosuppression

Induction therapy consisted of basiliximab (20 mg at day 0 and day 4). In kidney transplant recipients, in case of retransplantation or high immunological risk (defined as a panel of reactive antibodies of >50% or the presence of donor-specific anti-HLA antibodies), thymoglobulin at a dose of 1.5 mg/kg/day was given for 3–4 days. Maintenance immunosuppression consisted of tacrolimus, mycophenolate with or without steroids. In kidney transplant recipients, kidney biopsies were not performed by protocol, so that all biopsies were clinically indicated. In lung transplant recipients, bronchoscopy with bronchoalveolar lavage and protocol biopsies were performed at 2 and 4 weeks and at 3, 6, 12, and 24 months post-transplant and when clinically indicated. Liver transplant recipients did not undergo protocol biopsies.

All medical charts were reviewed in detail, and we collected data on demographic characteristics, immunosuppressive therapy, and episodes of rejection occurring within 12 months after immunization.

Vaccine

All patients received the trivalent-inactivated split-virion influenza vaccine Mutagrip® (Sanofi-Pasteur MSD, Switzerland), prepared from virus grown in the allantoic cavity of embryonal hens' eggs. In addition, patients vaccinated in 2009 received two doses (at 3-week intervals) of the influenza A H1N1/09 AS03-adjuvanted vaccine (Pandemrix; GlaxoSmithKline, Brendford, UK). This vaccine was composed of a split-inactivated A/California/07/2009 (H1N1)-derived strain of influenza virus containing 3.75 μg of hemagglutinin antigen. The AS03 adjuvant was composed of squalene (10.69 mg), DL-α-tocopherol (11.86 mg), and polysorbate 80 (4.86 mg).

Laboratory assays

Sera were tested before and 4–8 weeks after immunization for the presence of anti-HLA antibodies of IgG isotype using the Luminex solid-phase assay [Luminex LABScreen Mixed®] (Luminex Inc., Austin, TX, USA). This assay contains a panel of color-coded microbeads coated with multiple HLA antigens to identify class I and class II anti-HLA antibodies. Antigen–antibody complexes were identified using goat anti-human IgG conjugate coupled with phycoerythrin. In case of a positive screening result, the specificity of the anti-HLA antibodies was assessed using high-definition single LABScreen® Single Antigen class I or class II antigens. Test interpretation was performed by HLA Visual® software (One Lambda)

on the LABSCAN100™ flow cytometer (Luminex Inc., Austin, TX, USA).

Statistical analysis

We compared continuous variables with *t*-tests or nonparametric tests (Wilcoxon for two samples, and Kruskal–Wallis) when appropriate. We compared proportions with the chi-square test or the Fisher's exact test with a statistical significance level assigned at $P \leq 0.05$. All analyses were performed using PASW Statistics 18 (IBM Corporation, Armonk, NY, USA).

Results

Patient population

A total of 169 subjects were included in the study: 136 kidney, 26 lung, three liver, and four combined organ transplant recipients. One hundred and eight SOT recipients were included in the previous studies of influenza vaccination; 61 new patients vaccinated in the 2011–2012 influenza season were added to the cohort. Baseline characteristics of the patient population are shown in Table 1. The median interval between transplantation and vaccination was 28 months. Concerning the immunosuppressive regimens, 79% of patients had received basiliximab, and 13% of patients had received thymoglobulin as induction at the time of transplantation. More than 90% of patients were on a maintenance immunosuppression combining a calcineurin inhibitor (CNI) and an antimetabolite.

Development of anti-HLA antibodies

Five (2.9%) of 169 patients developed *de novo* anti-HLA antibodies. According to the type of transplant, the rate of *de novo* anti-HLA antibodies was 5 of 136 (3.7%) in kidney, 0 of 26 (0%) in lung, 0 of 3 (0%) in liver, and 0 of 4 (0%) in combined transplant recipients. Table 2 shows the clinical and immunological characteristics of the five patients with *de novo* anti-HLA antibodies. In three of the five patients, mean fluorescence intensity (MFI) was below 2000 (the established cutoff for positivity in our center), ranging from 506 to 972. One patient developed an anti-HLA class II (DR13) at 2082 MFI; anti-HLA screening test remained positive for class II antibodies during the following months, but without specificity. One patient, whose anti-HLA antibodies were not detectable 5 weeks after vaccination, developed a donor-specific antibody (DSA) class II (DQ7) at a MFI of 7997 8 months after vaccination, with concomitant biopsy-proven chronic active antibody-mediated rejection. Thus, overall, only one patient of 169 (0.6%) developed DSA after influenza vaccination.

Positive anti-HLA results were observed in 35 (21%) of 169 patients before vaccination (29 kidney, four lung, one liver, and one combined transplant recipients). Overall, 18 patients had anti-HLA class I antibodies and 25 had anti-HLA class II antibodies. In patients with pre-existing antibodies, no *de novo* DSA developed and median MFI was not significantly different before and after vaccination (6009 vs. 6283; $P = 0.73$, for anti-HLA antibodies class I, and 9131 vs. 8234; $P = 0.20$, for anti-HLA antibodies class II, respectively), as shown in Fig. 1. Of the five patients

Table 1. Baseline patients' characteristics.

Variable	All patients ($n = 169$)	Lung transplant recipients ($n = 27$)	Adjuvanted influenza A H1N1/09 vaccine ($n = 28$)
Recipient age; median years (IQR)	50 (39–60)	50 (31–59)	50 (41–58)
Recipient sex, M/F	109/60	14/13	14/14
Time from transplant; median months (IQR)	28 (10–71)	65 (28–83)	38 (13–101)
Organ transplant, *n* (%)			
Kidney	136 (80)	–	23 (82)
Lung	26 (15)	26 (96)	–
Liver	3 (1.8)	–	3 (11)
Combined	4 (2.4)	1 (4)	2 (7)
Retransplantation, *n* (%)	21 (12)	1 (4)	6 (21)
Induction therapy, *n* (%)			
Basiliximab	134 (79)	27 (100)	16 (57)
Thymoglobulin	22 (13)	0	6 (21)
None	13 (8)	0	6 (21)
Maintenance immunosuppression, *n* (%)			
Calcineurin inhibitor	163 (96)	26 (96)	28 (100)
MMF/MPA	155 (92)	23 (85)	20 (71)
mTOR inhibitor	5 (3)	1 (4)	0 (0)
Prednisone	125 (74)	26 (96)	16 (57)

MMA, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

Table 2. Clinical and immunological characteristics of the five patients with *de novo* anti-HLA antibodies after influenza vaccination.

No.	Age	Gender	Organ transplant	Retransplantation	Adjuvanted vaccine	Time from transplant to vaccination (months)	Anti-HLA antibodies (mean fluorescence intensity)	Donor-specific antibodies	Acute rejection (time from vaccination, days)
1	65	M	Kidney	No	Yes	9.4	B8 (839), B78 (805), A68 (506)	No	No
2	28	M	Kidney	No	No	8.3	DR9 (972), DR52 (926), DR15 (733)	No	No
3	54	M	Kidney	No	No	7.5	DR13 (2082), DR17 (1695), DR11 (1641), DR14 (1636), DR18 (829), DR52 (646)	No	No
4	47	F	Kidney	Yes	No	75.5	DR7 (693)	No	No
5	29	M	Kidney	No	No	24	DQ7 (7997)	Yes	Yes (233)

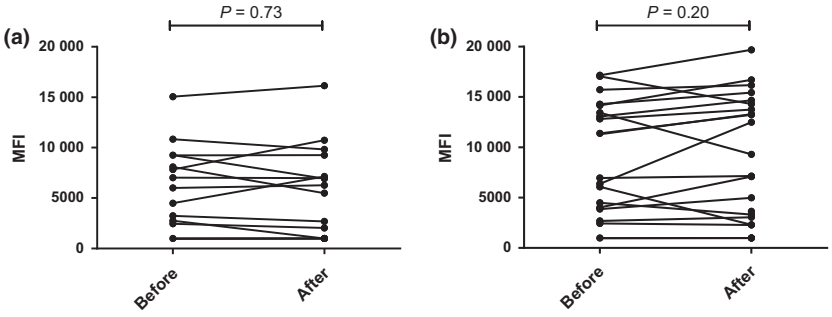

Figure 1 Evolution of anti-HLA antibody titers before and after influenza vaccination in solid organ transplant recipients in 35 patients with pre-existent anti-HLA antibodies (29 kidney, four lung, and two combined transplant recipients). (a) anti-HLA antibodies class I (n = 18). (b) anti-HLA antibodies class II (n = 25). Only the antibody with the highest mean fluorescence intensity titer was used for each patient in this comparison. Nine of these patients had received more than one organ transplant.

with pre-existent DSA, a difference in MFI was not observed after vaccination (P = 0.31). From the 21 patients who received more than one transplant, 8 of 21 patients (38%) had pre-existent anti-HLA antibodies and one patient (Table 2, patient #4) developed low-level *de novo* non-DSA anti-HLA antibodies (MFI 693).

Forty-four kidney transplant recipients were followed for more than one influenza season. Neither *de novo* DSA nor significant change in MFI of pre-existing anti-HLA antibodies was seen in these patients (data not shown).

Rejection episodes and allograft function

Five rejection episodes were documented within 12 months after vaccination, all in kidney transplant recipients. Two episodes of acute cellular rejection grade IB were diagnosed, respectively, four and 9 months after vaccination. One episode of mixed acute cellular and antibody-mediated rejection was seen in a noncompliant 22-year-old patient 2.5 months after vaccination. One episode of chronic antibody-mediated rejection was diagnosed 6 months postvaccination, after an episode of acute antibody-mediated rejection before vaccination. None of these four patients had *de novo* anti-HLA antibodies after vaccination. As previously mentioned, one patient developed an episode of chronic antibody-mediated rejection with development of *de novo* DSA 8 months after vaccination.

In kidney and combined transplant recipients (n = 140), mean serum creatinine levels were not significantly different before and 6 weeks after influenza vaccination (131 ± 43 μM vs. 131 ± 42 μM, respectively, P = 0.90). Data on lung function were available for 16 lung transplant recipients. There were no significant differences in the FEV1 before and after vaccination (median of 2.36 l [IQR 1.78–3.5] vs. 2.43 l [IQR 1.74–3.44], P = 0.89; respectively).

Adjuvanted influenza vaccine

In 2009, 28 patients received two doses of the AS03-adjuvanted influenza H1N1/09 vaccine and had stored sera available for determination of anti-HLA antibodies. Six patients had pre-existing anti-HLA antibodies. Only one patient developed *de novo* anti-HLA antibodies (Table 2,

patient #1). During the 12-month follow-up, one patient with pre-existing anti-HLA antibodies developed an episode of humoral rejection 182 days after vaccination.

Discussion

Some reports have suggested that influenza vaccination might trigger cellular and humoral alloimmunity, with an increased risk of allograft rejection [8,13]. However, the observed stimulation of alloimmunity was always transient without clear clinical consequences. Larger clinical studies showed no increased risk of allograft dysfunction or rejection after vaccination [10,11,16]. We analyzed this issue by investigating the influence of influenza vaccination on anti-HLA sensitization and allograft rejection. In our series of 169 SOT recipients, we did not observe a significant change in humoral alloimmunity after influenza vaccination. Only five patients (2.9%) developed *de novo* anti-HLA antibodies, of whom only one patient (0.6%) had *de novo* DSA. Our data are in concordance with the results of three other studies that analyzed postvaccinal anti-HLA antibodies in kidney and heart transplant recipients, where the incidence of *de novo* antibodies was very low [11,17,18]. For example, in a study by Broeders *et al.*, [18] the prevalence of anti-HLA class I and class II antibodies in 111 kidney transplant recipients was, respectively, 15% and 14% before adjuvanted influenza vaccination and 14% and 14% after vaccination.

Katerinis *et al.* [13] evaluated the humoral alloimmune response of multiple doses of influenza vaccine (one dose of seasonal influenza and two doses of AS03-adjuvanted H1N1 vaccine) in two independent cohorts of renal transplant recipients, showing a higher incidence of *de novo* anti-HLA antibodies as compared with a previous vaccination against seasonal influenza (15% vs. 6%). This observation raised the question whether the adjuvant contained in the vaccine, by enhancing innate and adaptive immune responses, could have caused an allostimulatory response. However, influenza infection itself (even asymptomatic) might also be held responsible for the antibody response, as the pandemic influenza vaccine was administered during widespread influenza infection. Of note, most anti-HLA antibodies in the study of Katerinis *et al.* were detected at low MFI (<2000) and disappeared after a 6-month follow-up period. In our study, only 1 of 28 patients having received the pandemic adjuvanted vaccine developed *de novo* anti-HLA antibodies, and this at low MFI and without any clinical significance [19,20].

The most important concern for clinicians is whether influenza vaccination can trigger allograft rejection. Anecdotal reports of viral infection-induced graft rejection were published in the precyclosporine era [21,22], but direct associations between vaccination and acute rejection have not been recently demonstrated. In our study, we did not observe a high rate of acute rejection during the 12-month follow-up period. Episodes of rejection occurred in renal transplant recipients without *de novo* anti-HLA antibodies after vaccination. One patient developed a chronic antibody-mediated rejection with appearance of *de novo* DSA 8 months after vaccination. However, this episode of chronic rejection is difficult to be ascribed to vaccination, as the patient had no anti-HLA antibodies before and 5 weeks after vaccination. While it is plausible to think that influenza vaccination may trigger some episodes of rejection in individual patients at high immunological risk, the significant higher rate of influenza complications (including acute rejection) in unvaccinated patients clearly indicates that the benefits of influenza vaccination in SOT recipients outweigh the potential risks [6].

Our study has several limitations. First, we did not have a control group of nonvaccinated patients to compare the incidence of the development of anti-HLA antibodies or acute rejection. However, in the context of a very low incidence of postvaccination *de novo* anti-HLA antibodies, it would have been difficult to obtain a sample size powered enough to show significant differences between groups. Second, the relatively modest sample size of our cohort and the short time from vaccination to the determination of anti-HLA antibodies might have underestimated the actual incidence of alloimmune responses following the administration of the influenza vaccine (due to a type 2 error), particularly regarding the development of subclinical DSA weeks or months after vaccination. Finally, the population included in the present analysis is somewhat heterogeneous, with different SOT recipients receiving different types of vaccine, which possibly detracts from the ability to assess the impact of each type of vaccine separately, particularly regarding the specific role of the adjuvanted vaccine in triggering alloimmune responses [13]. Nevertheless, we believe that the results presented here are relevant in view of some previous controversies in this field.

In conclusion, development of DSA or anti-HLA antibodies after influenza vaccination in our cohort of SOT recipients was a very rare event (0.6% and 2.9%, respectively) and no evidence was observed suggesting higher rejection rates after influenza vaccination. Thus, our findings indicate that influenza vaccination is safe and can continue to be recommended to all SOT recipients.

Authorship

PV, MP and OM: conceived and designed the study. Data collection was carried out by all authors. VA and RS: performed the assay. PV and OM: drafted the paper. All authors revised and approved the final manuscript.

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