Effects of Influenza Immunization on Humoral and Cellular Alloreactivity in Humans

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Background. Alloreactive T cells and anti-human leukocyte antigen antibodies mediate transplant injury. Environmental exposures, including vaccinations, may activate the alloimmune repertoire leading to accelerated allograft injury. To test whether vaccination impacts human alloimmunity, we analyzed humoral and cellular immune reactivity in subjects undergoing influenza vaccination.

Methods. We serially obtained blood samples from 30 healthy subjects and 8 kidney and 9 lung transplant recipients who received influenza vaccination, and from 20 healthy unvaccinated controls. We measured cellular and humoral anti-influenza responses, anti-human leukocyte antigen antibodies, and alloreactive T-cell immunity (interferon-γ ELISPOT) at 0, 2, 4, and 12 weeks after vaccination.

Results. Vaccination induced influenza-reactive humoral and cellular responses in control subjects and in transplant recipients. Only two of 30 vaccinated volunteers developed new alloantibodies, but none of the transplant patients. Vaccination also specifically and significantly augmented cellular alloimmunity based on reactivity to a panel of stimulators in both healthy subjects and in transplant recipients within 4 weeks of vaccination. The enhanced cellular alloresponse waned toward prevaccine levels by week 12.

Conclusion. Our findings newly demonstrate that influenza vaccination can have a significant impact on the potency of the alloimmune repertoire. Because the strength of the alloresponse influences long-term graft function, our results suggest that further investigation of alloimmune monitoring after vaccination is needed.

Keywords: T cell, Influenza, Vaccination, Allosensitization, Heterologous immunity.

(*Transplantation* 2010;89: 838–844)

T-cell- and antibody-mediated allograft injury continues to limit the long-term success of organ transplantation. Preformed anti-human leukocyte antigen (HLA) alloantibodies and primed donor-reactive T cells are particularly problematic because they resist standard immunosuppression and can rapidly engage effector functions without the need for in vivo reactivation (*1–4*). B-cell and T-cell sensitization to alloantigens may be induced on exposure to alloantigens from previous transplantation, pregnancy, or blood transfusions, but they can also be found in human transplant candidates who have never been knowingly exposed to HLA antigens, presumably due to cross reactivity to environmental antigens (*5*, *6*).

Emerging evidence from animal models and from select human studies indicates that alloreactive T cells can be activated by antigens derived from infectious pathogens that cross react with alloantigens, a phenomenon that has been termed heterologous immunity (*7–9*). In addition to direct cross reactivity between pathogens and alloantigens, pathogen-induced activation of the innate immune system (e.g., through toll-like receptor stimulation) can augment cytokine induction and up-regulate costimulatory molecule expression, thereby enhancing expansion and differentia-

This work was supported by NIH K23 RR 022956 (L.D.-I.), NIH/NIAID K23 5K23AI068824 (E.D.P.), and Clinical Trials in Organ Transplantation (NIH U01 AI63594; P.S.H.).

The authors declare no conflict of interest.

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L.D.-I. participated in research design, writing of the manuscript, statistical analysis, and performance and overall supervision of research; L.C. participated in writing of the manuscript, laboratory experiments, and statistical analysis; H.S., M.M., and K.K. participated in laboratory experiments; M.B. and D.S. participated in writing of the manuscript and interpretation of data; J.J.A., D.E.H., and R.F. participated in data interpretation, data analysis, and writing of the manuscript; and P.S.H. and E.D.P. participated in research design, data analysis and interpretation, and writing of the manuscript.

Received 19 October 2009. Revision requested 19 October 2009.
Accepted 29 October 2009.

ISSN 0041-1337/10/8907-838
DOI: 10.1097/TP.0b013e3181ca56f8

tion of the alloimmune repertoire regardless of the initially inciting antigen (*10–12*).

During the last decade, newer immunosuppressive medications have significantly decreased the rate of acute cellular rejection with the consequences of overimmunosuppression and the risk of infection (*13*). Viral infections, including influenza infection, occur at higher frequency after effective immunosuppression (*14*, *15*). As a consequence, systematic preventive strategies including empirical prophylaxis, routine screening, and vaccination are commonly used. Current recommendations urge annual influenza vaccination for solid organ transplant recipients, supported by evidence suggesting that influenza vaccination of transplant recipients induces effective humoral and cellular immune response against viral antigens without significant adverse events (*16–20*).

Although the benefits of vaccination are known, exposure to influenza antigens through vaccination could directly activate alloreactive T and B cells (heterologous immunity). Vaccination could also activate innate immunity, indirectly augmenting alloreactive memory T and B cells, which could translate into an increased risk for subsequent graft injury. Although controversial, the most recent studies suggest that vaccinations do not increase the risk of clinical acute rejection (*21–23*). However, the impact of vaccination on chronic graft injury remains unknown. This is particularly relevant because sustained increases of primed alloreactive T cells and or alloantibodies are associated with the development of chronic allograft injury (*24*, *25*). Therefore, it becomes essential to understand whether and how vaccination, particularly with a commonly used vaccine, influences the alloimmune repertoire both in transplant candidates and in immunosuppressed transplant recipients.

To test this, we studied the effects of seasonal influenza vaccination on anti-HLA antibodies by flow cytometry, and alloreactive T cells by the ELISPOT-based panel of reactive T cells (PRT) assay, in a cohort of healthy volunteers and in lung and kidney transplant recipients.

MATERIALS AND METHODS

Study Population

Patients and healthy volunteers who were offered influenza vaccination during the 2006 to 2007 and 2007 to 2008 seasons at the Cleveland Clinic and University Hospitals of Cleveland were considered for participation in this study. After institutional review board approval, enrolled subjects provided informed consent before participation. A total of 17 transplant recipients (nine lung transplant recipients and eight kidney transplant recipients) and 30 nontransplant subjects who opted to receive the vaccination and 20 nontransplant controls who refused vaccination enrolled in the study. No transplant recipient refused vaccination. All transplant recipients were on triple drug immunosuppression including prednisone, a calcineurin inhibitor, and an antiproliferative agent. Calcineurin inhibitor levels were recorded during the study for comparison. Volunteers did not have any medical conditions requiring immunosuppressive medications. Blood samples were collected just before vaccination, 2 weeks, 4 weeks, and at 12 weeks postvaccination in all subjects. In transplant recipients, graft function was monitored for 1 year after vaccination. Serum creatinine was used to assess graft function in kidney transplant recipients and forced expiratory volume at 1 sec was used in lung transplant recipients.

Vaccine

All participants received a 0.5-mL dose of commercially available trivalent split influenza vaccine (Fluzone, Sanofi-Aventis, Bridgewater, NJ) by intramuscular injection. The 2006 to 2007 and the 2007 to 2008 vaccines used the same antigens and each contained 15 μg of hemagglutinin of the following strains: A/Wisconsin/67/2005 (H3N2-like virus), A/New Caledonia/20/99 (H1N1-like virus), and B/Malaysia/2506/2004-like virus. Other components of the vaccine were also the same.

Alloreactive and Anti-Influenza T-Cell Detection

Peripheral blood samples were obtained in heparinized tubes at the specified time points. Aliquots of serum were stored at −70°C and used for antibody testing. Unfractionated peripheral blood mononuclear cells (PBMCs) were prepared and tested in INF-γ ELISPOT assays as previously described (*16*, *26–29*). The responder cells were tested in triplicate against medium alone (negative control), a panel of five HLA-typed allostimulator cells (using 100,000 B cells per well), and phytohemagglutinin (positive control). Results were depicted as the mean number of interferon (IFN)-γ spots per 200,000 recipient peripheral blood lymphocytes (PBLs) based on triplicate measurements in a given assay and against any given stimulator. The frequencies of alloreactive IFN-γ ELISPOTs used for analyses were obtained after subtracting those derived from nonstimulated wells ("background"). The frequencies of IFN-γ spots before vaccination were considered the baseline response of each subject to any particular stimulator. Postvaccination frequencies of IFN-γ spots against each of the five stimulators of the panel were assessed in relation to the baseline or prevaccination response (fold increase), and thus, each subject served as its own control. The response to the panel is based on the fold increase response at any given time point over the baseline. A response of at least 100% increase over baseline (doubling of frequencies) was considered a positive response. PRT assays were constructed based on the number of positive responses to each stimulator in relation to the total of five stimulators, that is, if a particular subject had at least doubling of frequencies at a determined follow-up time point in two of the five stimulators, this subject was considered to have a PRT assay of 40%. PRT values were calculated at 2 and 4 weeks with the highest of each response considered as a measure of the peak response. A 12-week PRT value was also calculated as a measure of residual response.

To assess the possibility of a nonspecific adjuvant stimuli from the vaccination as a causative of the postvaccination effect observed, we measured (1) IFN-γ secretion by T cells in response to media (background) in postvaccination time points in relation to baseline and (2) autologous IFN-γ secretion in four vaccinated subjects (two with high postvaccination alloresponse and two with no postvaccination response) by incubating each responder's PBMCs with self B cells cultured under the same stimulating conditions as the panel of allostimulators (*28*). Briefly, B cells were isolated using a neg-

TABLE 1. Population characteristics

	Unvaccinated volunteers (n=20)	Vaccinated volunteers (n=30)	Transplant recipients (n=17)	P
Female, n (%)	18 (90.0)	27 (90.0)	4 (23.5)[a]	<0.01
Age (yr), mean (SD)	39±10	36±12	49±12[a]	<0.01
White	15 (75.0)	24 (80.0)	13 (76.5)	0.90
h/o pregnancies, n (%)	7 (35.0)	12 (40.0)	3 (17.6)	0.69
h/o blood transfusions, n (%)	1 (5.0)	1 (3.3)	9 (52.9)	<0.01
Months since transplant, median (minimum–maximum)	NA	NA	25 (9–147)	NA

NA, not applicable.

ative selection magnetic separation for B-cell ($CD19^+$) enrichment (StemCell Technologies, Vancouver, Canada), stimulated by CD40L transfected fibroblasts and IL-4 until they reached their log phase of growth, and then stored frozen for use in control experiments. B cells were checked for ability to allostimulate before use in syngeneic controls.

Antiviral cellular responses were tested in IFN-γ ELISPOT assays using antigen directly derived from the vaccine at a dilution of 1:10,000. This dilution was found to provide the best read out from titration experiments (data not shown). A positive response was also determined based on the relation of baseline anti-influenza reactive PBLs to follow-up time point responses. Fold increase over baseline frequencies were compared among the different cohorts at either 2 or 4 weeks and at 12 weeks postvaccination.

Alloantibody and Antiviral Antibody Detection

Serum anti-HLA antibody was determined by flow cytometry using HLA class I and class II antigen-coated latex beads (FlowPRA Screening Test, One Lambda Inc., Canoga Park, CA). The antibody testing was performed according to instructions supplied by the manufacturer. A positive panel reactive antibody (PRA) was defined as greater than 10% reactivity for either class I or class II antigen. A doubling of the PRA percentage at any time point after vaccination from baseline or a 10% increase was considered a positive alloantibody response, and we used single antigen beads to assess for specificity of the alloantibody for positive responses.

Anti-influenza antibodies against each of the strains contained in the vaccine were measured by hemagglutination assays at the Glennan Center Laboratory at East Virginia Medical School (Norfolk, VA). Postvaccination titers at 4 weeks were compared with baseline titers, and the mean fold increase was calculated to assess vaccination response. Titers were not measured at 2 weeks or 12 weeks postvaccination. Either a baseline titer to each strain of at least 1:40 or a fourfold increase in titers over baseline was considered appropriate immunization response to influenza. Seroprotection was defined as having a baseline or follow-up anti-influenza titer of at least 1:40 and seroconversion as having at least a fourfold increase in titers from baseline (*30*).

Statistical Analysis

All analyses were performed using JMP 7.0 (SAS Institute Inc., Cary, NC). Values are shown as mean±SD, median and percentiles when data were not normally distributed, and percentages. Categorical variables were compared using the chi-square test or Fisher's exact test when appropriate. Comparison of mean values was tested using the Student's *t* test for independent samples (two tailed), and median comparison was performed using the Wilcoxon/Kruskal-Wallis test. Changes over time compared with baseline were calculated using matched pair Student's *t* test and confirmed by Wilcoxon sign rank test in those cases where data were nonparametrically distributed. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of the study cohorts are shown in Table 1. Transplanted patients were older, more likely to be male and more likely to have received prior blood transfusions compared with the healthy subjects.

Influenza Vaccination Induces Viral Reactive Immunity in Normal Subjects and Transplant Recipients

For humoral reactivity to viral antigens (anti-influenza antibodies), we used ELISA, and we found seroprotective titers to at least one influenza strain in 13/30 individuals (Fig. 1A). Because many antigenic epitopes are shared among viruses from year to year, previous vaccination and or influenza disease is anticipated to induce detectable immunity in a minority of subjects before vaccination. After vaccination, we found seroconversion or seroprotection in 24/30 individuals ($P<0.05$ vs. prevaccination). We similarly noted a significant increase in the percentage of seroconversion in transplant recipients after vaccination (1/17 prevaccination vs. 14/17 postvaccination, $P<0.05$). In contrast, in the unvaccinated subjects, we found no significant change in the prevalence of seroprotection (8/20 prevaccination vs. 10/20 postvaccination, P=NS).

For cellular reactivity to the vaccine antigens, we used IFN-γ ELISPOT (Fig. 1B). As we found for humoral immunity, the frequency of influenza-reactive IFN-γ producers increased significantly within 4 weeks postvaccination in the healthy subjects and the kidney transplant recipients (Fig. 1B and C; $P<0.01$ by matched pair analysis—Wilcoxon/Krustal-Wallis test—in each comparison). Vaccination was less effective at increasing the influenza reactivity in lung transplant recipients than in kidney transplant recipients (1/9 lung transplant recipients showed doubling of the baseline ELISPOT

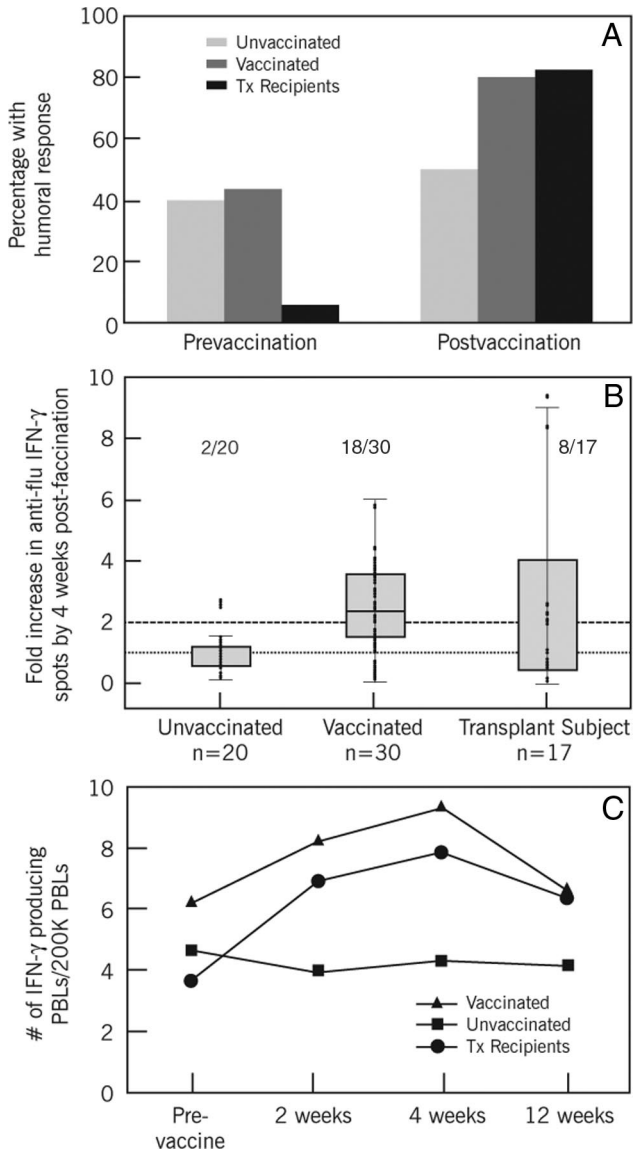

FIGURE 1. Humoral and cellular responses to influenza vaccination. (A) Humoral immune response to influenza vaccination. Prevaccination represents the percentage of each group with hemagglutination titers more than 1:40 to any of the three strains of influenza at baseline. Postvaccination represents a titer of more than 1:40 or a fourfold increase in hemagglutination response at 4 weeks after vaccination. (B) Fold increase in anti-influenza interferon (IFN)-γ spots by 4 weeks after vaccination (*P* value among three groups of <0.03 by analysis of variance and <0.01 by Wilcoxon sign rank test). (C) Cellular response to influenza vaccination as measured by ELISPOT during the study period for each group (*P* value nonsignificant between both vaccinated subgroups for any time point).

response vs. 4/8 kidney transplant recipients), perhaps due to significantly higher tacrolimus levels (mean levels of 10.1 and 5.3 ng/dL in lung and kidney recipients, respectively) ($P<0.001$). At 12 weeks postvaccination, we found a sustained increase in the frequency of influenza-reactive IFN-γ–producing PBMCs compared with the prevaccine values in the vaccinated cohort, although on average, the strength of the responses waned compared with the peak values noted at 2 to 4 weeks.

In contrast to the findings in vaccinated subjects, we noted no change in the frequency of influenza-reactive PBMCs in the unvaccinated individuals over 12 weeks (Fig. 1B and C, P=NS by matched pair analysis). Together, these data indicate that influenza vaccination induces humoral and cellular influenza-reactive immunity in the overwhelming majority of transplant recipients taking maintenance immunosuppressive medications (Table 2).

Influenza Vaccination and Anti-HLA Antibody

At baseline, we observed that the median PRA was less than 10% for each group. Less than 22% of those subjects about to be vaccinated had either a class I or class II PRA of more than 10% and less than 3% (none of the transplant patients) were sensitized as defined by a PRA of more than 80%. After vaccination, 2 of the 30 healthy subjects and none of the transplant patients developed stronger flow PRA values. Two subjects increased their class II PRA greater than 10% that were sustained during the 12-week study period. Although in one subject anti-HLA antibodies were not detected by single antigen beads, the other subject demonstrated a significant increase in detectable antibodies to multiple HLA antigens (DR11, DR13, DR16, DR51, and DR103).

Influenza Vaccination Is Associated With an Augmented Frequency of Primed Cellular Alloimmunity

We compared the strength of the responses to the PRTs assays prevaccination and postvaccination. Figure 2 depicts the percentage of individuals in each group who developed at least a twofold increase in alloreactivity to each of the stimulators at 2 to 4 weeks (A) or 12 weeks (B). A higher percentage of vaccinated subjects demonstrated increased responses to each of the allostimulators compared with unvaccinated subjects by either 2 or 4 weeks postvaccination ($P<0.01$ for stimulators 1–4 and $P=0.18$ for stimulator 5, Fig. 2A). We found that the responses in vaccinated volunteers exceeded the responses in vaccinated transplant subjects at this time point, but it was only statistically significant ($P=0.01$) for stimulators 2 and 4, whereas it was not statistically significant for the other three stimulators. The increased alloresponses were maintained in a subset of individuals for at least 12 weeks after vaccination (Fig. 2B).

We calculated the overall response to the panel of allostimulators as the proportion of positive responses (doubling from baseline) to the each member of the panel of five stimulators. Figure 3 depicts these results at 2 or 4 weeks (peak response) and at 3 months postvaccination (final response). Vaccinated subjects demonstrated a higher response to the panel independent of the cutoff (number of positive responses) used ($P<0.01$ for all cutoffs except for PRT-100 where $P=0.28$; Fig. 3). Transplant recipients also demonstrated an increase in alloreactivity after vaccination, although the proportion responding was less than in healthy vaccinated volunteers. Interestingly, although the cellular response seemed to be less evident in lung transplant recipients than in kidney transplant recipients, the PRT response was comparable among the two cohorts (2/9 lung transplant recipients had a PRT response to 40% of the panel vs. 2/8 kidney transplant recipients). In conjunction, 4/19 (~24%) trans-

TABLE 2. Humoral and cellular response to influenza vaccination

Response to influenza vaccine	Unvaccinated, n=20	Vaccinated, n=30	Transplant (lung/kidney), n=17 (9/8)	*P*
Cellular and Humoral	0 (0%)	14 (47%)	6 (3/3) (35%)	<0.01
Humoral only	10 (50%)	10 (33%)	8 (4/4) (47%)	0.04
Cellular only	2 (10%)	4 (13%)	2 (1/1) (12%)	0.9
Neither	8 (40%)	2 (7%)	1 (1/0) (6%)	<0.01

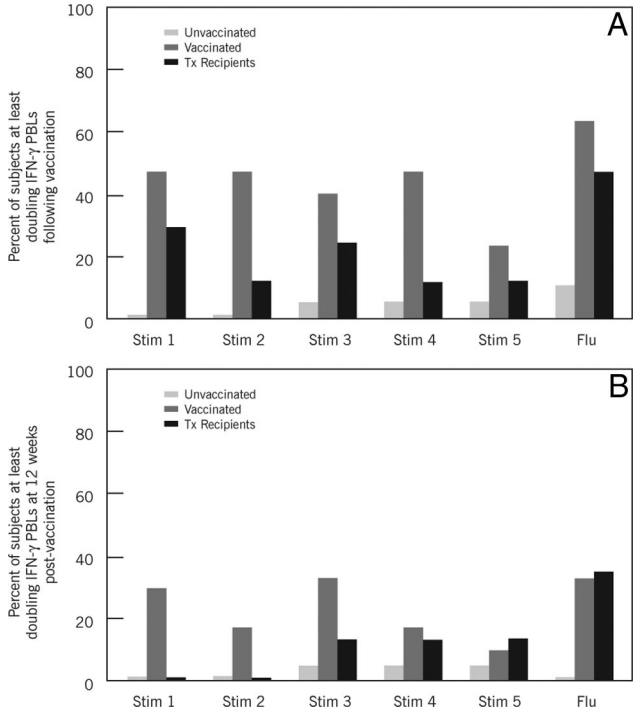

FIGURE 2. Percent of unvaccinated controls, vaccinated controls, and transplant recipients with at least a doubling of the baseline alloresponses to each stimulator at 2 to 4 weeks (A) and 12 weeks (B) postvaccination.

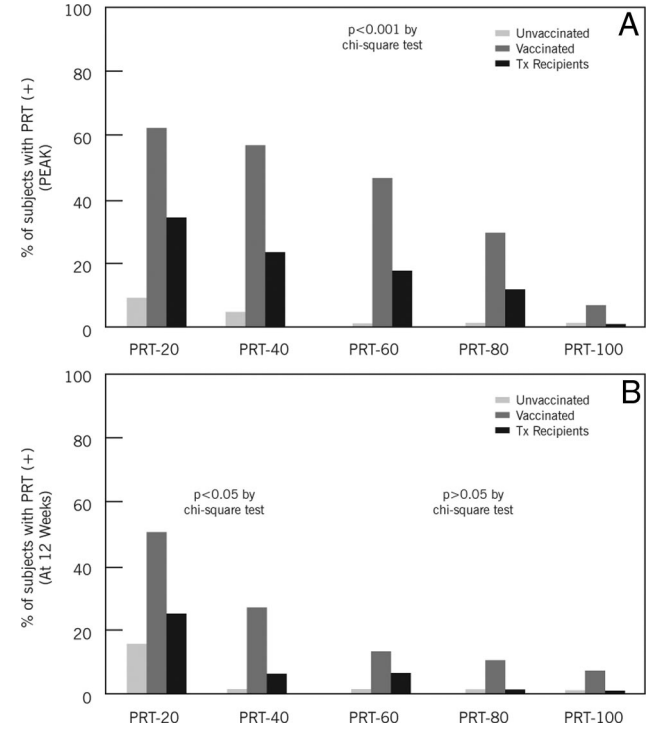

FIGURE 3. Panel of reactive T cells using different cutoff values for each subgroup at 2 to 4 weeks postvaccination (A) and 12 weeks postvaccination (B).

plant recipients had a reactivity to at least 40% of the panel (Fig. 3B). Three of the four transplant recipients who reacted to two of five stimulators of the PRT panel (PRT-40) were the same who doubled the cellular response to the influenza antigen after vaccination. The remaining transplant recipient (lung) with reactivity to the flu developed a new reactivity to one stimulator (PRT-20) and two of the kidney transplant recipients with reactivity to the influenza antigen did not develop any panel reactivity (PRT negative). Similar patterns of antiviral and PRT panel reactivity were observed in the vaccinated controls (Table 3).

Analogous to the cellular responses to viral antigens, the alloresponses for the entire vaccinated cohort declined by 12 weeks postvaccination (Fig. 3B; matched pair analysis between mean PRT at 2–4 weeks vs. PRT at 12 weeks postvaccination, $P<0.01$ by Wilcoxon Signed-Rank). Clinical evidence of graft rejection was not observed in any of the transplant patients within 3 months of vaccination.

Control studies revealed no change in spontaneous IFN-γ secretion (media wells) for any of the samples within each group postvaccination. To further assess for nonspecific stimulation by B-cell–secreted factors, we performed ELISPOT assays using syngeneic, self-B cells as stimulators. We tested PBLs from two vaccinated subjects without prevaccine or postvaccine alloreactivity and two vaccinated subjects with detectable alloimmune responses that were augmented postvaccination. Regardless of the change in alloresponses after vaccination, the responses to self-B cells did not change significantly postvaccine in any subject, indicating that the vaccination altered alloimmunity but not self reactivity (data not shown).

DISCUSSION

We present novel data indicating that vaccination against influenza results in temporal changes in cellular alloimmune responses. This information is important as it suggests that (1) vaccination can affect alloimmunity, and (2) antiviral and anti-HLA immune monitoring could be applicable to transplanted patients. Alloreactivity in general is detrimental to transplant outcomes, with alloreactive effector or memory T cells being

TABLE 3. Antiviral and anti-HLA cellular response agreement

	Unvaccinated volunteers (n=20)	Vaccinated volunteers (n=30)	Transplant recipients (n=17)
Anti-influenza positive and PRT-40 positive	1 (5)	16 (53.3)	3 (17.6)
Anti-influenza negative and PRT-40 negative	18 (90)	10 (33.3)	10 (58.8)
Anti-influenza positive and PRT-40 negative	1 (5)	3 (10)	3 (17.6)
Anti-influenza negative and PRT-40 positive	0 (0)	1 (3.3)	1 (5.8)

Values are expressed as n (%).
HLA, human leukocyte antigen; PRT, panel of reactive T cells.

central mediators of the immune-mediated injury to the graft (*1*, *3*, *4*). When present, these cells have been shown to impair graft tolerance and to accelerate rejection in animal models (*4*, *7*). In humans, pretransplant and posttransplant circulating alloreactive T cells have also been associated with graft rejection (*2*, *6*, *24*, *27*, *28*). These cells are commonly found in subjects with previous exposures to alloantigens including pregnancy, blood transfusion, or transplantation; however, cellular alloimmunity can also be found in subjects without any prior history of such events, (*2*, *6*, *29*) suggesting that other responsible mechanisms of allosensitization exist. Hypothesized factors that could cause cross reactivity with alloantigens are viral infections or vaccinations; however, there are no studies in humans evaluating this theory.

In this study, we show that vaccinated subjects—both healthy volunteers and transplant recipients—exhibited increased cellular responses to commonly expressed human alloantigens after vaccination, whereas the unvaccinated cohort did not. The effects of influenza vaccination on alloreactivity achieved its maximum effect 2 to 4 weeks after vaccination, with attenuation in the alloimmune response by 3 months postvaccination in most of the studied subjects. The response in vaccinated healthy subjects exceeded that response in transplant recipients, likely due to the nonexposure to immunosuppressive drugs in the healthy cohort. In addition, lung transplant recipients, who had significantly higher tacrolimus levels during the study period, showed decreased responses to the influenza antigen compared with kidney transplant recipients.

In animal models, several mechanisms may explain the development of HLA reactive T cells in the absence of direct exposures to alloantigens. In heterologous immunity alloreactive T cells can be generated by incomplete allelic exclusion leading to allospecific T cells. A second mechanism is when T-cell receptors can directly cross react to viral antigens due to molecular mimicry (*8*). It is also plausible that several nonallospecific stimuli, such as vaccinations, temporarily enhance preexistent alloreactivity through a bystander effect. Because response to one stimulator predicted response to other allostimulators, our data suggest that the observed increase in T-cell alloreactivity may be due to a nonspecific reactivation of a variety of memory T-cell clones and not specifically those able to cross react with viral antigen. Conversely, the absence of self-responses and increased alloreactivity in those transplanted subjects who also showed evidence of anti-influenza cellular reactivity suggest that cross reactivity cannot be disregarded. However, an important point is that irrespective of the underlying mechanism leading to enhanced alloreactivity, identification and closer follow-up of transplant subjects during the perivaccination period with the use of immune monitoring tools that detect antivaccine and anti-HLA cellular reactivity may be justifiable.

Clinically, the concern for the practicing healthcare professional relates to whether vaccination can trigger clinical rejection. Anecdotal reports of influenza vaccination-induced graft rejection were common during the precyclosporine era, (*31–34*) but direct associations between vaccinations and acute graft rejection have not been recently described (*17*, *22*, *35*, *36*). Increased alloreactivity did not translate directly into episodes of acute rejection in our cohort; however, influenza vaccination was associated with increased cellular alloreactivity, an important event that is known to participate in the pathogenesis of chronic rejection (*37*, *38*). The response attenuated within 3 months of vaccination but repeated exposure to vaccination could allow for the accumulation of alloreactivity increasing the risk for subsequent chronic immune-mediated graft injury. Further evaluation of the persistence or recurrence of enhanced alloreactivity with repeated exposures is needed. Our data further suggest that the effects of influenza vaccination on alloreactivity are variable and unpredictable, but the use of noninvasive immune assays permitted us to detect and follow the alloimmune response during its peak and subsequent attenuation of the response.

Common practice at many centers delays vaccination at least 3 months after transplantation, when the state of heavy immunosuppression early posttransplant has declined to allow an effective antiviral immune response. It could additionally be hypothesized that because the risk of acute graft rejection is highest early after transplantation, stimulation of the immune system by vaccination may precipitate early graft rejection. These assumptions could be extended to the pretransplant period when transplantation is anticipated within 1 to 3 months to avoid the peak of cellular alloreactivity postvaccination. However, antiviral and anti-HLA cellular and humoral immune monitoring may aid in determining vaccination efficacy and state of alloimmunity in the peritransplant period (immediately prior or after transplantation) or in any circumstances in which immunosuppression is drastically modified, like in minimization regimens or treatment of rejection episodes.

Our study is limited by the lack of dedicated mechanistic studies to help understand the pathophysiologic processes behind the reported observations; however, this study provides the first human evidence that viral vaccinations may play a role in alloimmunity. Moreover, whether temporarily enhanced alloreactivity in humans by any mechanism triggered by viruses indeed have any impact on HLA-immunity

mediated graft injury needs to be better elucidated. Our current work is also limited by the evaluation of a single vaccination, and therefore, whether alloreactivity can also be affected by other organisms or vaccinations is unknown, nor did we specifically study HLA specificities of the cellular alloreactivity. However, studies in animal models have demonstrated that heterologous immunity may occur with a variety of pathogens (*7*, *9*, *39*, *40*). Clinical implications of other infectious diseases on the alloimmune response in transplant recipients remain uncertain and require further investigation. Further, the study did not evaluate repeated episodes of vaccination, limiting the understanding of repeated stimulation on alloreactivity. Finally, sample size was small to elucidate whether this increase in alloreactivity relates to clinical graft rejection and follow-up longer than 3 months postvaccination until the alloresponse leveled off was not pursued.

In conclusion, influenza vaccination elicits an appropriate antiviral humoral and cellular response in most transplant recipients; however, a significant proportion of vaccinated subjects concomitantly demonstrated an increase in alloreactivity against a broad panel of allogeneic stimulators. In the future, antiviral and anti-HLA immune monitoring may be potentially clinically useful for surveillance of circulating alloreactive T cells in solid organ transplant candidates and recipients after influenza vaccination and may aid in personalization of care.

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