Human Vaccines & Immunotherapeutics 8:12, 1850–1853; December 2012; © 2012 Landes Bioscience

Persistence of influenza vaccine-induced antibody in lung transplant patients and healthy individuals beyond the season

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Keywords: influenza vaccine, lung transplantation, antibody, waning, persistence, immunosuppression

Abbreviations: HIA, hemagglutination inhibition assay

Background: The timing of influenza vaccination and susceptibility to re-circulating virus in the population is influenced by the persistence of seroprotection. Immunosuppressed transplant patients are known to have lower antibody response rates than healthy individuals, but acceptable antibody concentrations are achieved. The duration of this seroprotection beyond a single season has not been evaluated in either healthy or immunosuppressed populations.

Results: Seroprotection rates for influenza A and B strains at one year following immunization were 100% for lung transplant and healthy controls. Rates at two years for the influenza A strains were 65–74% for lung transplant vs. 77–100% in healthy controls. Rates for influenza B strains two years following immunization were 27–50% for lung transplant vs. 16–38% in healthy controls. (Fisher's exact test; not significant for between group comparisons; $p < 0.05$ for between season comparisons).

Methods: Influenza antibody concentrations against viruses no longer included in the vaccine were measured in serum by hemagglutination inhibition assay annually following vaccination of 73 lung transplant participants and 27 healthy controls. Seroprotection was defined as a titer of ≥ 1:40 and was compared between groups over the measured term using Fisher's exact tests.

Conclusions: Vaccine-induced antibody persistence appears to be influenced more by the vaccine virus strain than the immune status of the vaccinated individuals. Seroprotection rates are high 12 mo following influenza vaccination but wane over the second year, particularly for influenza B viruses. Annual influenza immunization is indicated, even for healthy individuals and even when the vaccine viruses do not change.

Introduction

Every year influenza infection causes significant morbidity and mortality in the general population making it a serious public health concern. Among those at highest risk for complications from infection are the immunocompromised. Effective influenza immunization that confers protection throughout the season is critical for lung transplant recipients because in addition to aggressive immunosuppressive therapy, infection directly affects the transplanted organ.[1-4] Studies of influenza vaccine response rates in lung transplant patients show generally lower antibody concentrations, but acceptable influenza vaccine response rates compared with healthy individuals.[5-7] However, little is known about the persistence of influenza vaccine-induced antibody concentration in either healthy or immunosuppressed populations. Influenza antibody concentrations persist at seroprotective levels (defined as antibody concentrations at least 40 hemagglutination units[8]) up to a year post vaccination.[9,10] However, we could find no information regarding persistence beyond one year. We hypothesized that the high rates of persisting influenza vaccine-antibody in immunosuppressed lung transplant individuals would match the rates of persisting vaccine-antibody in healthy individuals.

Results

The transplant participants were statistically significantly older than the healthy participants (**Table 1**). The median time since transplant is reported.

There was no difference in seroprotection rates between healthy individuals and lung transplant participants. The rates of seroprotection in transplant participants never dropped significantly lower than that of healthy individuals for any viral strain in any season (**Table 2**). Although rates of seroprotection were maintained equally across both groups, seroprotection rates against influenza B viruses seemed to decrease much more rapidly than the seroprotection against influenza A types in both groups (**Table 2**).

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Submitted: 04/03/12; Revised: 07/26/12; Accepted: 08/05/12
http://dx.doi.org/10.4161/hv.21735

Table 1. Demographics

	Healthy	Lung transplant	p value
Age in years (mean ± SD)	44.7 ± 11.2	51.7 ± 11.9	< 0.005*
Male sex (%)	11/27 (41%)	35/73 (48%)	Not significant**
Time since transplant (months)			
2004 (median, range)	—	37 (2–156)	
2005 (median, range)		44 (3–168)	
2006 (median, range)		56 (15–180)	
2007 (median, range)	—	65 (5–192)	

*Students t test, **Chi square test.

We considered the effect of annual influenza immunization on the antibody concentration to influenza viruses no longer contained in the vaccine. We collected pre- and post-immunization sera from many participants as part of the original study so we could measure change in antibody concentration with immunization with other influenza vaccine viruses. An increase in pre-immunization to post-immunization antibody concentrations would indicate that antibody cross-reactivity existed. Of the participants vaccinated in 2006 with the H1N1 influenza strain, 29% (n = 16 of 56) experienced significant boosting of the H1N1 antigen in 2007 defined as greater than or equal to 2-fold increase. In 2008, only 2% (n = 1 of 62) experienced boosting. Of the participants vaccinated in 2004 with the H3N2 influenza strain, 9% (n = 6 of 69) experienced boosting in 2005 and 14% (n = 3 of 22) in 2006. No participants experienced boosting in 2007. Of the participants vaccinated with the influenza B strain in 2005, no participants experienced boosting in 2006 or 2008.

Discussion

The duration of seroprotection from influenza vaccines does not differ between immunosuppressed lung transplant patients and healthy individuals. Seroprotection persists at a high level for approximately two years after immunization. The waning of antibodies seems to be more dependent on the virus strain included in the vaccine than the age difference between the two groups or immunological status of the vaccinated individual. This variation is not a new observation and is supported by other studies.[11] The fact that we measured antibody persistence beyond one season is unique to our study. Our findings indicate that antibody to influenza B virus is least persistent after vaccination waning from 100% after the first year to between 50–16% the second year. This differs from seroprotection levels conferred by influenza A vaccine strains. All H1N1 and H3N2 strains tested maintained seroprotection levels of antibody well over 50% even two years after immunization. However, seroprotection rates were low by 2.5 and 3 y post-immunization.

We assumed a linear rate of decay in seroprotection for our analyses. In reality, it is probable that antibody does not decay linearly. There have been studies in healthy individuals showing that a short antibody waning period occurs immediately after immunization with linear decay up to 1 y post-vaccination.[12] The fact that seroprotection rates that are not significantly different between groups implies that any spontaneous drop off of antibody or any nonlinear decay occurs in participants of both groups at similar points in time. To increase the value of our study, it would be useful to take more blood draws between our time points to clear up any discrepancies between the two groups' seroprotection levels that are invisible to us with the limited time points available.

Universal annual seasonal influenza vaccination is recommended so re-exposure to potentially cross-reactive vaccine viruses reflects clinical practice. Our boosting data show variability in the change in antibody concentrations upon exposure to different vaccines viruses. Also based on antibody changes, we defined influenza infection based on serologic evidence which may lead to underestimating the true rate of infection.[13]

The mean age of the transplant participants was statistically significantly higher compared with the mean age of the healthy group. Although studies have shown that people over the age of 65 y have a decreased immune response to influenza vaccine,[14,15] age is not a clinically important confounder in this study because the oldest healthy participant was 63 y old. Only five transplant patients in all 73 participants were over 65 y of age, the oldest being 73 y of age. However, generalization of these results to pediatric and elderly populations is not supported.

Occasionally, influenza vaccine composition does not change between seasons. Some immunization experts questioned the need for repeat immunization when the vaccine is the same.[16] Although our seroprotection rates are quite high at the end of two years, clinicians should not be willing to accept seroprotection rates in the 30% range as we showed for influenza B. Our results should persuade vaccine providers to continue to recommend annual influenza immunization for both the healthy population and immunocompromised patients, even when the vaccine viruses do not change as antibody protection falls by the end of the second season.

Seroprotective antibody concentrations to influenza A viruses persist at high rates for almost two years after immunization with no difference between healthy individuals and immunosuppressed lung transplant patients. The specific viral strain included in the vaccine seems to influence antibody persistence more than the immune status of the individual. Both healthy and immunosuppressed individuals should receive influenza vaccines annually to maintain high rates of seroprotection.

Our study has several limitations. We may have been unable to show differences between groups because of the relatively small sample size. The transplant subject group was older than the healthy control subjects, but these differences are not clinically significant from an immunological standpoint. Studies finding a decreased antibody response to influenza vaccine with age include subjects over age 65 y.[14,15] Using the serological diagnosis of influenza infection allows us to detect unreported and undiagnosed influenza infections. Serological diagnosis is useful for a study of influenza vaccine antibody persistence where waning of antibody concentrations are the main outcome, influenza infections may

Table 2. Persistence of influenza vaccine-induced antibody

Vaccine virus	1 y		2 y		2.5 y		3 y	
	Healthy	Transplant	Healthy	Transplant	Healthy	Transplant	Healthy	Transplant
2006–07 A/New Caledonia H1N1	15/15 (100%)	46/46 (100%)	9/9 (100%)	34/46 (74%)	–	–	6/9 (67%)	11/38 (29%)
2007–08 A/Solomon Islands H1N1	23/23 (100%)	46/46 (100%)	18/23 (78%)	30/46 (65%)	8/21 (38%)	12/34 (35%)	–	–
2004–05 A/Wyoming H3N2	18/18 (100%)	57/57 (100%)	14/18 (78%)	35/52 (67%)	–	–	4/15 (27%)	7/51 (8%)
2007–08 A/Wisconsin H3N2	22/22 (100%)	47/47 (100%)	17/22 (77%)	31/47 (66%)	6/21 (29%)	16/35 (46%)	–	–
2004–05 B/Shanghai	22/22 (100%)	40/40 (100%)	8/21 (38%)	20/40 (50%)	–	–	1/18 (6%)	7/31 (23%)
2007–08 B/Malaysia	19/19 (100%)	45/45 (100%)	3/19 (16%)	12/45 (27%)	1/17 (6%)	5/34 (15%)	–	–

All between group comparisons not statistically significant by Fisher's exact test. All between season comparisons statistically significant different by Fisher's exact test except control 2006 A/New Caledonia year 1 vs. year 2 vs. year 3 and control and transplant 2007 B/Malaysia year 2 vs. year 2.5

have been missed because they did not cause an increase in relevant influenza antibody concentrations, but these non-vaccine influenza infections will not have an effect on the study outcome. Another assay for protection from influenza infection could be considered. Neutralization assays have the advantage of measuring effective antibodies. However, the assay is subject to higher variability than hemagglutination inhibition assays are.[17]

Materials and Methods

As part of a five-year study of influenza antibody response in lung transplant patients,[5,9] we measured antibody concentrations to influenza viruses that were eliminated from the seasonal influenza vaccine over time. Twenty-seven healthy individuals were enrolled over the course of the study (**Table 3**). Those who dropped out were replaced. In 2004 influenza vaccine production was limited resulting in allocation to healthcare workers and other high risk populations, thus the inclusion of hospital employees was necessary. Seventy-three lung transplant patients from the University of Wisconsin Hospital and Clinics were enrolled over the course of the study. All individuals were vaccine responders at one year post- immunization. All lung transplant patients were maintained on immunosuppressive regimens consisting of prednisone, a calcineurin inhibitor and an antiproliferative agent. Each participant received the seasonal trivalent inactivated influenza vaccine each year of participation. Individuals were excluded if they had an allergy to eggs, refused the vaccine or did not maintain seroprotection after one year after vaccination defined as antibody titer ≥ 1:40 to any individual vaccine virus.[8] Any study participant who showed a 4-fold increase in antibody concentration was considered to show serological evidence of an influenza infection and the data after the infection were not included for the rest of the study. However, the data from participants who received influenza diagnoses during a season were included if they showed no serological evidence of infection with any of the relevant influenza strains being studied.

Table 3. Study participation by year

	Controls	Attrition	Transplant	Attrition
2004–05	n		n	
1 y	24		58	
2 y	23	1 no sample	58	
3 y	22	1 no sample	53	2 deaths 3 no sample
2006–07				
1 y	15		46	
2 y	9	6 no sample	46	
3 y	9		38	3 deaths 5 no sample
2008–09				
1 y	23		47	
2 y	23		47	
2.5 y	21	2 no consent	34	7 no consent

The study protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board, and all participants gave their written consent to participate.

Serum was collected from participants prior to and between two to four weeks after annual trivalent inactivated influenza immunization for five consecutive seasons. A span of 2–4 weeks was acceptable for the post-immunization blood draw because it has been shown that antibody responses to influenza vaccination are similar at two and four weeks post-immunization.[18] Influenza antibody concentrations were measured by hemagglutination inhibition assay (HIA) in samples taken at baseline and two to four weeks after immunization. The laboratory staff performing HIA using standard microtiter techniques was blinded as to participant status. Briefly, antibodies present in the human serum inhibit influenza virus-induced agglutination of guinea pig red

blood cells. Serial dilutions of the sera are made. Titrated influenza antigen is incubated with the serum dilutions for 30 min. Guinea pig red blood cells are added and incubated for 45 min. The dilution of serum that no longer inhibits hemagglutination is the influenza antibody titer. Antibody concentrations that were below the lower limit of detection (< 1:10) were assigned a concentration of 1:1.[19]

SPSS V.20 was used for all statistical analyses. Because this study was part of another study of influenza vaccine response in lung transplant patients, the number of participants who were eligible for this study was pre-determined and changed during the follow-up time. With a sample size of 20 healthy controls and 58 transplant patients, we had 80% power to detect a seroprotection rate that was 64% of the control group. We assumed that the seroprotection rate would decrease over time so we used a 90% estimate for seroprotection in the control group. Student's t-test was used to compare mean ages between the healthy and lung transplant participants. Chi square test was used to evaluate any differences in sex distributions between the groups. Fisher's exact tests were used to compare seroprotection rates between the groups and between seasons.

Disclosure of Potential Conflicts of Interest

J.J.S., K.R.R. and J.J.M.M. have no conflicts of interest to disclose. M.S.H. is a member of speakers' bureau for Merck Vaccines.

Acknowledgments

This research study was funded in part by NATCO Research Grant Award.

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