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Decreased T cell responses to influenza vaccination in patients with heart failure

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Abstract

Objectives—Influenza infection leads to increased mortality rates during winter months for patients with heart failure. Data support increased influenza rates in patients with HF despite widespread immunization. In this study, we measured T cell immune responses in patients with heart failure (HF) compared to healthy controls (HC) following influenza vaccination using the *trans vivo* delayed-type hypersensitivity (DTH) assay, a method which mimics physiologic conditions.

Design—Prospective, single center study

Setting—University hospital and research laboratory

Patients—Eighteen patients with stable chronic HF maximized on optimal HF therapies and 16 HC.

Intervention—Participants were immunized with the 2006/2007 trivalent inactivated (killed) influenza vaccine. Blood was drawn prior to and 2–4 weeks following influenza vaccination, in addition to between January and April following vaccination.

Measurements and Main Results—Peripheral blood mononuclear cells (PBMC) were isolated from blood drawn prior to and following immunization. PBMC were mixed with influenza vaccine antigens (A/H1N1, A/H3N2, and B/Malaysia), and injected into the footpads of SCID mice. The resulting swelling is an index of human T cell sensitization. Humoral responses were measured in serum with hemagglutination inhibition assay. Participants in the HF group demonstrated a less vigorous T-cell-mediated immune response to A/H3N2 (HF: median 62.5 μ m; healthy controls: median 87.5 μ m, unadjusted $p=0.031$, age adjusted $p=0.006$ for comparison between groups). Responses to A/H1N1 were not significantly different between the groups (HF: median 56.3 μ m; healthy controls: median 75 μ m, $p=0.11$). Finally, responses to B/Malaysia were not different between groups (HF: median 62.5 μ m; healthy controls: 75 μ m, $p=0.47$). All participants mounted an antibody response to the influenza vaccine.

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Conclusion—HF pts demonstrated reduced T cell responses to influenza vaccine compared to HC, as demonstrated by a lower response to the newest vaccine antigen. Lower T cell responses may indicate that HF patients are at increased risk for influenza infection.

Keywords

heart failure; influenza; immune function; vaccine

Introduction

Chronic heart failure (HF) predisposes to influenza infection and its complications. Excess mortality observed during winter months in individuals with HF attributes to influenza.¹ Vaccination against influenza infection decreases cardiac related hospital admissions, acute HF exacerbations, and all cause mortality.² Despite widespread influenza vaccination programs, overall influenza-related hospitalization and death rates are rising, particularly in patients with cardiac disease.¹

Older adults and persons with cardiac disease or other co-morbidities and treatments that render them immune-compromised are at greater risk for influenza infection despite vaccination due to reduced antibody and cell mediated vaccine responses.^{3, 4} In HF, an upregulated sympathetic nervous system⁵ may decrease immune response via activation of beta₂-adrenergic receptors (β_2 -AR), leading to reduced cytokine production necessary for vaccine immune response.^{6, 7} Therefore, it is logical that patients with HF demonstrate rising influenza-related morbidity and mortality as compared to those without cardiac disease, potentially due to up-regulated adrenergic pathways.

Most adults develop both humoral antibody and T-cell immune responses to influenza vaccination, indicating that both type 1 (Th1) and type 2 (Th2) responses are made following influenza immunization.^{8–10} The most widely accepted indicators of influenza vaccine immune response are seroconversion and seroprotection, referring to antibody titer changes to one of the three vaccine viral strains. However, post-vaccination antibody titers as a measure of vaccine efficacy and protection against influenza illness in older adults do not measure cell-mediated immunity that is particularly affected by disease and increasing age.¹¹ Previous research of immune response in HF, including our own, used in vitro methods for measurement of T-cell-mediated responses, which can be highly variable.^{12, 13} In this study, we measured T-cell responses to influenza vaccination using *trans vivo* delayed-type hypersensitivity (DTH), a novel method to investigate T cell function and sensitization under physiologic conditions. We hypothesized that patients with HF will mount less vigorous T cell responses to influenza vaccination compared with healthy individuals.

Methods

Patients

Participants were a subset derived from a previous study of immune responses to influenza vaccination in HF patients who agreed to an additional blood draw. We studied patients with HF in addition to healthy individuals (controls). Eligible HF participants had systolic or diastolic dysfunction (documented by echocardiogram in previous 6 months) with American College of Cardiology(ACC)/American Heart Association(AHA) Stage C, New York Heart Association (NYHA) Functional Class I, II or III HF. All patients with HF were on stable medical therapy for HF for at least 30 days, including target or maximally tolerated doses of angiotensin converting enzyme (ACE) inhibitors and beta adrenergic blockers, when appropriate. Healthy controls and HF patients with a history of allergic reaction to influenza

vaccine, allergy to egg products, or moderate to severe acute febrile illness were excluded. None of the participants had immunological disorders, and none were taking immunosuppressive medications prior to or during the study. The protocol was approved by the University of Wisconsin institutional review board. All participants provided written informed consent in accordance with established guidelines for the protection of human subjects.

Study Protocol

This was a prospective, open label study in 16 healthy individuals and 18 individuals with established HF on stable medical therapy followed at the University of Wisconsin Hospital Advanced Heart Disease Program. The primary outcome variable was the difference in T cell mediated immune responses to influenza vaccination between patients with HF and healthy controls.

The influenza vaccine viral strain content changes annually to contain virus antigens from what are anticipated to be the three most commonly circulating strains in a given year. The three types of virus strains included are A/H3N2, A/H1N1, and B type, classified based on viral surface proteins. For the 2006/2007 season, the vaccine contained A/New Caledonia/20/99 (H1N1)-like virus; A/Wisconsin/67/2005 (H3N2)-like virus, and B/Malaysia/2506/2004-like virus.

Participants received the 2006–07 trivalent inactivated (containing killed virus) influenza vaccine intramuscularly during October through December of 2006. Participants underwent phlebotomy prior to and two to four weeks following vaccination for antibody titer measurement, and again between January and April following vaccination to obtain lymphocytes for T-cell responses measured with *trans vivo* DTH. The timing of these samples ensured adequate immune response to the vaccine after immunization.^{14–17} Importantly, measurement of antibody responses to influenza vaccine are similar at 2 and 4 weeks following immunization.¹⁸

For *trans vivo* DTH, peripheral blood mononuclear cells (PBMC) were isolated and washed. Cells were counted and resuspended in phosphate buffered saline (PBS). Six million PBMC were mixed with antigen or control and injected in a final volume of 35 μ L into footpads of anesthetized (isoflurane) SCID mice (CB.17 mice with severe combined immunodeficiency; campus breeding colony). Patients' lymphocytes alone, with tetanus toxoid (positive control, Sanofi Pasteur, Swiftwater, PA), or with influenza antigens (24 hemagglutination inhibition units from each viral strain of A/H1N1; A/H3N2; and B; Centers for Disease Control and Prevention, Atlanta, GA) were injected into murine footpads using a 0.5 mL syringe with a 28-gauge needle. Six mice were used to measure DTH responses for each subject. We measured T cell responses to individual influenza antigens alone and in combination. The resulting footpad swelling is an index of human T cell sensitization. Measurements of footpad thickness were performed with a dial thickness gauge (Mitutoyo, Aurora, IL) before and 18–22 hours after injection. Background swelling due to lymphocytes and buffer was subtracted to determine antigen specific responses.

Hemagglutination inhibition assay (HIA) was used to measure influenza antibody concentrations following immunization. A protective antibody response to influenza vaccine equates to an antibody titer equal to or greater than 1:40. Seroconversion following influenza immunization is defined as a fourfold increase in antibody concentrations.^{19, 20} HIA was performed in duplicate using standard microtiter techniques. Briefly, influenza virus-induced agglutination of guinea pig red blood cells was inhibited by antibodies present in the human serum. Serial dilutions of the human sera were made. Titrated influenza antigen was incubated with the serum dilutions for 30 minutes. Guinea pig red blood cells

(50 μ l of 0.5% in phosphate buffered saline) were added and incubated for 45 minutes. The dilution of serum that no longer inhibits hemagglutination was the influenza antibody titer.^{15, 16, 21}

Data Analyses

To identify potential differences between HF and control participants, we compared baseline demographics using t-tests for continuous variables and chi-Square tests for categorical variables. The primary study outcome variable for statistical analysis was differences in DTH reactivity (measured through influenza specific swelling of murine footpads) after influenza vaccination between participants with HF and healthy controls. Assuming a 25 μ m difference in murine footpad swelling between the two groups, 15 participants were needed in each group to achieve 80% power at the 0.05 significance level. We examined the influenza specific swelling elicited by lymphocytes from healthy individuals and that of HF patients. We compared the differences of footpad swelling elicited by influenza antigens (all three strains combined and individually) between healthy and HF groups with a Wilcoxon rank sum test. Finally, we compared the seroprotection (HIA >40) and seroconversion (>4 fold increase) rates in each group using chi square or fisher's exact tests. Data were analyzed on the intent-to-treat principle.

General linear regression was used to examine associations between age, prior vaccination, and seroconversion as well as T-cell responses to individual antigens alone and in combination between HF and healthy control groups. Subgroup analyses for participants with HF included examination of associations between age and ejection fraction upon T-cell-mediated immune responses. Separate linear regression models were constructed for each variable.

Results

Participants

Eighteen HF patients (4 females, 14 males) and 16 healthy controls (7 females, 9 males) were enrolled, with a mean age of 54 ± 14 years (median 54 years, range 26 – 81 years) for HF participants, and 47 ± 9 for healthy controls (median 49 years, range 29 – 65, $p=0.06$ for comparison between groups). Among the HF group, mean ejection fraction was $40 \pm 9\%$ (median 35, range 25%-55%), and patients were NYHA functional classes I (N=13), II (N=4), and III (N=1). Other baseline characteristics for the HF group are shown in Table 1. No adverse reactions were reported following influenza vaccination.

T-cell responses to influenza vaccine

T-cell data are summarized in table 2. The mean time from immunization to blood draw for DTH did not differ significantly between HF participants and healthy controls (112 days versus 107 days, respectively). There were no differences in responses to tetanus toxoid (positive control) between individuals with heart failure and healthy controls (HF: median 68.8 μ m; healthy controls: median 62.5 μ m, $p=0.48$). Participants in the HF group demonstrated a less vigorous T-cell-mediated immune response to A/H3N2, as shown in table 2 (HF: median 62.5 μ m; healthy controls: median 87.5 μ m, unadjusted $p=0.031$, age adjusted $p=0.006$ for comparison between HF and healthy controls). Responses to A/H1N1 were not significantly different between the groups (HF: median 56.3 μ m; healthy controls: median 75 μ m, $p=0.11$). Finally, responses to B/Malaysia were not statistically different between groups (HF: median 62.5 μ m; healthy controls: 75 μ m, $p=0.47$ for comparison between HF and healthy controls). No differences were noted between groups in responses to the combination of all three vaccine viruses (HF: 75 μ m; healthy controls: 87.5 μ m, $p=0.22$). Within the HF group, responses to A/H3N2 were associated with age (correlation

coefficient: 0.51, $p=0.02$) but not with ejection fraction. Differences in influenza vaccine responses between groups did not associate with prior vaccination history.

Antibody-mediated responses to influenza vaccine

All participants demonstrated seroprotection to the influenza vaccine. There was a trend toward lower seroconversion rates among HF participants compared to healthy controls (9 of 17 HF participants compared with 14 out of 16 healthy controls), a difference short of statistical significance ($p=0.057$, fisher's exact).

Discussion

In this study, we compared T cell responses to influenza vaccination in patients with heart failure versus healthy individuals using *trans vivo* DTH. The *trans vivo* DTH is a method which uses the mouse footpad as the site of T-cell-mediated immune reaction.²² PBMC and specific antigens are injected into the footpads of naïve, immunodeficient mice. The swelling that occurs within 24 hours of injection serves as an index of human T cell sensitization. No swelling occurs or histopathology appears in response to human PBMC or antigen alone. Therefore, the swelling when PBMC and recall antigen are injected together indicates T-cell-mediated activity. The *trans vivo* DTH is antigen specific; footpad swelling occurs only when the subject has been exposed to the antigen previously.^{17, 22, 23} The *trans vivo* DTH method mimics physiologic conditions for immune responses and potentially results in less variability compared to in vitro methods of T-cell response measurements.

Participants with HF exhibited lower T-cell-mediated responses to the A/H3N2 vaccine viral strain. The composition of the trivalent influenza vaccine changes with each influenza season. The A/H3N2 strain was the newest strain to be incorporated into the vaccine for the 2006/2007 season. The most widely accepted measures that indicate appropriate immune responses are based on antibody titers to one of the three vaccine viral strains; seroprotection is defined as an antibody titer ≥ 40 , and seroconversion entails a 4 fold increase in antibody titer. However, T-cell immune responses to influenza vaccine are known to wane with increased age and presence of chronic health conditions. Previously, investigators found that pre and post vaccination antibody titers alone did not distinguish between HF participants who subsequently developed influenza illness and those who did not.¹¹ Despite antibody titers indicative of seroprotection, participants with HF who subsequently developed laboratory diagnosed influenza illness (LDI) exhibited differing T-cell responses compared to participants who did not develop LDI. Specifically, individuals with LDI exhibited a lower IFN γ :IL-10 ratio.¹¹ Likewise, we also detected lower IFN γ :IL-10 ratios in patients with heart failure when compared to healthy individuals.¹³ This immune response phenotype suggests a shift from a Th1 to Th2 T-cell response, and may be a potential indicator for high influenza infection risk despite immunization.

Similar to these findings, we found decreased T-cell immune responses, evidenced by decreased DTH activity to the A/H3N2 vaccine strain in HF participants versus healthy controls. This suggests that for patients with HF, and potentially for those with other chronic diseases, it may be advantageous to measure T-cell immune responses in addition to antibody titers. Of note, we did not detect differences in T-cell immune responses to the A/H1N1 and B/Malaysia strains, suggesting that patients with HF were able to mount an appropriate response to previously presented vaccine antigens, but not when presented with a new vaccine antigen, as compared with healthy controls. The fact that responses to the positive control tetanus toxoid did not differ between groups also suggests that disparities in immune responses in patients with heart failure vary based on previous antigen exposure. Interestingly, our previous work showed that antigen specific antibody responses also differed between patients with heart failure and healthy individuals.²⁴ Importantly, antibody

responses to the A/H3N2 antigen were reduced in patients with heart failure despite similar rates of seroconversion and seroprotection as healthy controls. Examining individual antigen specific responses may therefore yield relevant information about vaccine responses beyond seroconversion and seroprotection rates. Our results suggest that patients with HF may rely on immunologic memory for mounting appropriate antibody-mediated immune responses to an antigen, whereas healthy individuals are able to mount a vigorous immune response upon exposure to a new antigen. It is also of note that as a group, participants with HF were only mildly symptomatic, had a mean ejection fraction of 40%, and were optimally treated with target doses of both ACE inhibitors and beta blockers. Our study suggests that even patients with mild heart failure may not be adequately protected against influenza infection despite annual vaccination. Studies examining the benefits of influenza immunization in patients with cardiovascular disease are favorable. However, future studies should explore additional or alternate influenza vaccination strategies for patients with heart failure.

Several limitations to our study need to be considered. First, immune responses to influenza vaccination are known to decrease with age, and our healthy control group trended younger than our HF group. Declining antibody responses to influenza vaccine have been observed in the elderly,^{25, 26} however both groups in our study had mean and median ages well below 65 years. Moreover, we adjusted for age statistically and the association of HF with reduced T-cell responses to A/H3N2 remained significant. Additionally, study participants had mildly symptomatic, stable heart failure. The generalizability of our results to a wider group of patients, especially those with more advanced HF, is unknown. Second, we did not find statistically significant differences in seroconversion between groups. However, our study was designed to detect differences in T-cell responses, so it is possible that we were not powered to capture differences in seroconversion rates. Due to our small sample sizes, it is also possible that differences in antibody-mediated immune responses did exist between study groups for the A/H1N1 and B-type vaccine viral strains. As such, antigen specific differences in immune responses should be explored more closely in a larger cohort. Last, we were unable to correlate differences in immune responses between the study groups to subsequent rates of influenza infection. A much larger sample size would be necessary to detect differences in influenza infection rates between two populations, particularly immunized populations. Future work should address the issue of individual strain vaccine-induced T-cell responses and clinical correlations with influenza infection rates more specifically.

In conclusion, patients with heart failure demonstrated decreased T-cell immune responses to the A/H3N2 viral strain compared with healthy controls, despite similar rates of seroprotection and seroconversion. The *trans vivo* DTH method is a useful technique to measure physiological T-cell immune responses in patients with HF.

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Table 1

Baseline Characteristics

Variable	HF Participants (N=18)	Healthy Controls (N=16)	P-value
Age (yrs)	54 ± 14	47 ± 9	0.11
Men, n (%)	14 (78)	9 (56)	0.18
White/black/Hispanic	17 / 0 / 1	16 / 0 / 0	0.92
Prior vaccination	15 / 16 (94%)	12 / 16 (75%)	0.14
NYHA FC I/II/III	13 / 4 / 1	-	
LVEF (%)	40.0% ± 9%	-	
Background therapy	-	-	
ACE inhibitor / ARB	18 (100%)	-	
Beta blocker	18 / 18 (100%)	-	
Furosemide	6 (33%)	-	
Digoxin	7 (44%)	-	
Spironolactone	6 (33%)	-	
Statin	10 (63%)	-	
Aspirin	9 (56%)	-	

* Data are mean ± SD unless otherwise indicated.

Table 2

Differences in antibody and T-cell responses between HF and healthy controls

	HF		Healthy Controls		P value[#]
Seroprotection Ab > 40 HAU	17 / 17 (100%)		16 / 16 (100%)		1
Seroconversion 4-fold increase	9 / 17 (53%)		14 / 16 (88%)		0.057
Trans vivo DTH responses	Median	Mean	Median	Mean	
A/H3N2 (A/Wisconsin)	62.5	63.9 ± 34.3	87.5	87.5 ± 24.6	0.031, 0.006*
A/H1N1 (A/New Caledonia)	56.3	56.9 ± 29.8	75	79.7 ± 39.2	0.11
B-type (B/Malaysia)	62.5	65.3 ± 37.0	75	74.2 ± 33.1	0.47
All 3 vaccine viruses	75	74.3 ± 43.8	87.5	85.6 ± 29.0	0.22

[#] Wilcoxon rank-sum

* P value after adjustment for age