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Brief report

Delayed-type hypersensitivity and hepatitis B vaccine responses, in vivo markers of cellular and humoral immune function, and the risk of AIDS or death

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ABSTRACT

Background: Delayed-type hypersensitivity (DTH) test responsiveness is associated with HIV disease progression; however it is unknown whether other immune markers, such as hepatitis B virus (HBV) vaccine seroresponse, also predict HIV outcomes.
Methods: Eligible participants received HBV vaccine after HIV diagnosis, had non-anergic DTH testing at the time of last HBV vaccination, and available post-vaccine HBV antibody responses. The risk of progression to AIDS or death from the time of last HBV vaccination was evaluated.
Results: Of 369 eligible participants with non-anergic DTH responses, 148 (40%) were HBV vaccine responders. In a multivariate model adjusted for age, CD4 count, viral load, and number of vaccinations, HBV vaccine non-responders had an increased risk of progression to AIDS or death (HR 1.81; 95% CI, 1.03–3.19).
Conclusions: HBV vaccine seroresponses were independent of DTH responses which suggest that non-response to HBV vaccine is not solely due to cell-mediated immune dysfunction in HIV-infected persons.

1. Introduction

The natural history of HIV infection results in a progressive decline in immune function and, if untreated, ultimately leads to the development of Acquired Immunodeficiency Syndrome (AIDS) and death. The advent of potent, well-tolerated antiretroviral therapy (ART) has significantly improved life expectancy in HIV-infected patients [1]. Despite the successes of modern ART regimens, both AIDS and serious non-AIDS events can occur, even at relatively preserved CD4 cell counts [2–4]. In addition to measurement of CD4 counts, other clinically relevant immune correlates are necessary to better understand HIV pathogenesis and predict disease progression.

Delayed-type hypersensitivity (DTH) testing is a tool that can be used to evaluate cell-mediated immunity (CMI) in clinical settings [5]. Cutaneous anergy to recall antigens is a predictor of HIV disease progression independent of viral load (VL) and CD4 count [6,7]. Whether markers for other aspects of immune function also predict HIV outcomes over and above DTH has not been completely studied. We previously demonstrated that hepatitis B virus (HBV) vaccine responsiveness can also be used to predict future AIDS events or death [8]. Although HBV vaccine is a T-cell dependent antigen, a positive seroresponse to HBV vaccine requires other aspects of immune function including antigen presentation of the peptide-based vaccine as well as B-cell activity [9–11]. We evaluated the relationships between the humoral response to HBV vaccine, CMI by DTH responses, and progression to clinical AIDS and death in the U.S. Military HIV Natural History Study (NHS).

2. Methods

The NHS is a prospective observational cohort comprised of more than 5500 military members, dependents, and beneficiaries with HIV-1 infection that are followed at 7 military medical treatment facilities in the U.S. since 1986. Participants enrolled were age 18 or older and provided signed, written consent in this IRB-approved study and are evaluated approximately every 6 months.

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Table 1
Characteristics of participants with non-anergic DTH test results at the time of last HBV vaccination by vaccine response category.

Characteristic	*N*	Anti-HBs < 10 IU/L *N* = 221	Anti-HBs ≥ 10 IU/L *N* = 148	*P*
Age (years)	369	31 (27–36)	31 (26–37)	0.76
Gender, male	369	202 (91)	124 (84)	0.03
Ethnicity	369			0.85
Caucasian		96 (43)	59 (40)	
African American		98 (44)	66 (45)	
Other		27 (12)	23 (16)	
CD4 count (cells/μL)	360	451 (318–613)	573 (418–737)	<0.001
CD4 count category (cells/μL)	360			<0.001
<200		23 (10)	6 (4)	
200–499		103 (47)	49 (33)	
≥500		90 (41)	89 (60)	
HIV VL (log_{10} copies/mL)	231	3.33 (2.30–4.38)	2.30 (2.21–3.85)	<0.001
HIV VL category (copies/mL)	369			<0.001
≤400		37 (17)	46 (31)	
>400		85 (38)	63 (43)	
Unknown		99 (45)	39 (26)	
Number of vaccinations	369			0.09
1–2		102 (46)	55 (37)	
≥3		119 (54)	93 (63)	
On ART at vaccination	369	182 (82)	102 (69)	<0.01
Year of vaccination	369			<0.001
<1996		134 (61)	58 (39)	
≥1996		87 (39)	90 (61)	
Follow-up (years)	369	5.4 (2.5–10.1)	6.7 (3.0–11.2)	0.16
Total (patient-years)		1463	1071	
Total number of AIDS or death events	369	68	17	<0.001

DTH, delayed-type hypersensitivity; HBV, hepatitis B virus; anti-HBs, antibody to HBV surface antigen; VL, viral load; ART, antiretroviral therapy.
Data are number (%) or median (interquartile range) unless otherwise specified.

In this retrospective study, participants were required to be HBV vaccine naïve prior to their HIV diagnosis with no history of HBV infection. Participants were also required to have received at least one dose of HBV vaccine and have DTH testing results at the time of last vaccination. Participants were classified as either vaccine responders or non-responders based on antibody to HBV surface antigen (anti-HBs) ≥10 or <10 IU/L, respectively. Since anti-HBs can wane ≥2 years after vaccination in HIV-infected adults [12], only participants with measured anti-HBs between 1 and 12 months after last vaccination were included to prevent misclassification. DTH testing was performed according to standardized NHS protocols as previously described [6,13,14]. A total of 0.1 mL of each antigen was applied intradermally to the forearm by Mantoux method and a positive test was defined as ≥5 mm of induration after 48 h. The most recent antigens and concentrations used per protocol consisted of tetanus toxoid (Lederle 1.6 Lf/mL; 1:100 dilution), mumps (Connaught, 40 CFU/mL), trichophyton (Holister-Stier, 1:500 dilution), and candida (Walter Reed Army Institute of Research, 200 PNU/mL). Participants received a panel of 3–4 antigens, with the majority receiving 3 antigens as trichophyton was removed from the market. DTH responses were categorized by the number of positive skin tests: anergic (0), partial anergic (1), or non-anergic (≥2) as previously described [13]. The subgroup with non-anergic DTH responses was further studied for clinical outcomes based on HBV vaccine seroresponse.

HBV vaccine non-responder and responder characteristics were compared by chi-squared or Fisher's Exact tests. AIDS outcomes were defined by 1993 Centers for Disease Control and Prevention criteria, with the exception of CD4 count [15]. Vaccine responders were followed from the time of their last HBV vaccination to the development of AIDS, death, or last study visit. Risk of progression for the combined endpoint of AIDS or death from the time of last HBV vaccination was evaluated with multivariate Cox regression and time to AIDS or death was assessed by Kaplan–Meier survival methods.

3. Results

A total of 507 NHS participants vaccinated from 1986 to 2005 met inclusion criteria, of which 65 (12.8%) had anergic DTH responses, 73 (14.4%) had partially anergic DTH responses, and 369 (72.8%) had non-anergic DTH responses. The proportion with a positive HBV vaccine response did not differ by DTH category: 31%, 30%, and 40% for anergic, partially anergic, and non-anergic, respectively ($P = 0.13$). Participants with non-anergic DTH responses were further investigated for HIV disease progression based on HBV vaccine seroresponse after last vaccination (Table 1). A greater proportion of participants were classified as non-responders (59.9%) compared to responders (40.1%), but demographic characteristics including age, gender, and race/ethnicity were similar between subgroups. Most participants in both groups were treated with ART at time of last vaccination and received ≥3 vaccine doses. At the time of last HBV vaccination, the median CD4 count and VL for non-responders compared to responders was 451 cells/μL (IQR 318–613) versus 573 cells/μL (IQR 418–737; $P < 0.001$) and 3.33 versus 2.30 log_{10} copies/mL, respectively ($P < 0.001$ for both). VL at last vaccination was unavailable for 138 (37.4%) of study participants since clinical VL assessments were not routinely performed prior to 1996.

Participants were followed for a median of 5.4 years (IQR 2.5–10.1) in the non-responder group and 6.7 years (IQR 3.0–11.2) in the responder group for a total of 1463 and 1071 person-years, respectively ($P = 0.16$). In participants with non-anergic DTH responses, 85 (23%) progressed to AIDS or death, including occurrence in 68 (31%) HBV vaccine non-responders and 17 (11%) responders ($P < 0.001$). In preliminary models, gender, year of vaccination, and receiving ART at vaccination were not significantly associated with AIDS or death risk. The risk of progression to AIDS or death for HBV vaccine non-responders and responders was assessed by multivariate analysis adjusted for age, CD4 cell count, HIV VL, and number of vaccine doses (Table 2). HBV vaccine non-responders were found to have an increased risk of progression to

Table 2
Final age-adjusted multivariate model for risk of progression to AIDS or death among those with non-anergic DTH test results.

Characteristic	HR (95% CI)	*P*
CD4 count category (cells/μL)		
<200	9.10 (4.66–17.75)	<0.001
200–499	2.11 (1.20–3.71)	0.01
≥500	Referent	
HIV VL category (copies/mL)		
≤400	0.13 (0.03–0.55)	<0.01
>400	0.66 (0.33–1.35)	0.26
Unknown	Referent	
Number of vaccinations		
1–2	1.70 (1.07–2.69)	0.02
≥3	Referent	
HBV vaccine response		
Anti-HBs <10 IU/L	1.81 (1.03–3.19)	0.04
Anti-HBs ≥10 IU/L	Referent	

DTH, delayed-type hypersensitivity; HR, hazard ratio; CI, confidence interval; VL, viral load; HBV, hepatitis B virus; anti-HBs, antibody to HBV surface antigen.
Gender, year of vaccination, and receiving ART at vaccination, were all not significantly associated with AIDS or death risk in preliminary models.

AIDS or death (HR, 1.81; 95% CI, 1.03–3.19). The time to AIDS or death was significantly greater for HBV vaccine responders compared to non-responders (Fig. 1; $P < .001$).

4. Discussion

In a large cohort of HIV-infected adults, we found that most participants with non-anergic DTH responses to recall antigens did not demonstrate protective seroresponses to HBV vaccine. We also observed that HBV vaccine response status was a predictor of subsequent AIDS or death among those with non-anergic DTH responses. These findings suggest that non-response to HBV vaccine is not entirely due to CMI dysfunction in individuals infected with HIV, but other immune deficits likely contribute to the lack of anti-HBs response after vaccination.

As an in vivo marker of CMI, DTH responsiveness is enhanced at higher CD4 cell counts. For example, one study showed that responses to 2 or more recall antigens was 86% for those with CD4 >400 cells/μL compared to only 45% for those <400 cells/μL [10]. However, DTH responsiveness has also been shown to be a predictor of HIV disease progression in untreated individuals independent of CD4 cell count [6,7,16]. A previous study in our cohort showed that the total number of reactive skin tests (0, 1, or 2–4) stratified patients according to survival time and progression to AIDS [13]. In the current study, we observed that in HIV-infected patients with non-anergic DTH responses, a marker for more favorable CMI, seroresponses to HBV vaccine also had prognostic value. Although DTH testing has long been used in clinical practice for various purposes, such as screening for latent tuberculosis infection, DTH methods are not routinely used in HIV medicine. HBV vaccine seroresponses may be of greater practical use since HBV vaccine is recommended for all HIV-infected persons and anti-HBs, a validated marker for HBV vaccine response, is routinely measured in clinical practice [17,18].

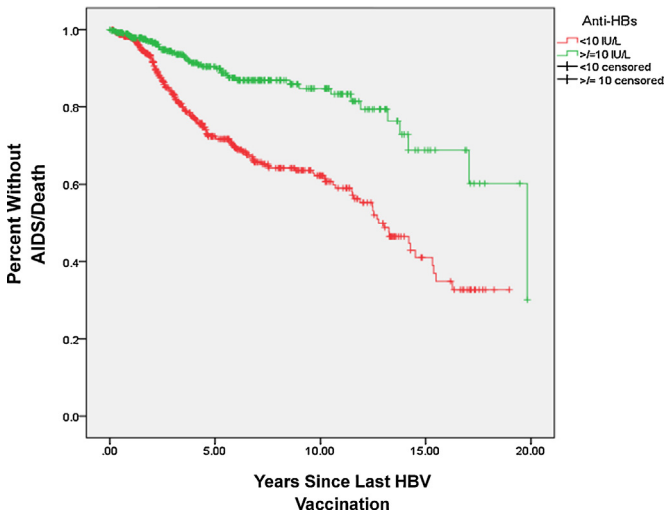

Fig. 1. Time to AIDS or death for those with non-anergic DTH test results by HBV vaccine response ($P < 0.001$ by log rank).

HBV vaccine response, a surrogate for humoral immune function, may be used in a similar manner as DTH response to help identify other host or viral factors that influence HIV disease course and potential response to specific therapies. The effect of HIV on CMI has been utilized in several prediction models to assess the severity of disease and response to therapy by evaluating CD4 cell count, total lymphocyte count, T-cell subtypes, and DTH response in addition to viral load as independent risk factors [8,13,14,19–21]. In a previous study in our cohort, a greater likelihood of HBV vaccine response was demonstrated in those with higher CD4 cell counts at last vaccination, use of ART, and receipt of ≥3 doses of HBV vaccine [21]. HBV vaccine seroresponse was also associated with progression to AIDS or death, even in those with CD4 counts >500 cells/μL. Since DTH responsiveness is improved at higher CD4 counts, the observation in the current study that HBV vaccine seroresponse remains predictive of HIV disease progression in those with non-anergic DTH testing is consistent with prior findings. Although HBV vaccine is a T-cell dependent antigen, only 40% of HBV vaccine responders were non-anergic by DTH testing which suggests that additional immune factors contribute to the development of anti-HBs response.

As future efforts continue to shed new light on the immunologic relationships in the pathogenesis and progression of HIV infection, HBV vaccine seroresponses may serve as a useful tool for predicting HIV disease progression in addition to other clinical laboratory makers such as CD4 count and VL. Identification of additional factors that affect the innate and adaptive immune components could help tailor treatments, guide future therapies, and provide additional data to predict the course of disease for individuals infected with HIV.

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