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Serological Immunity to Adenovirus serotype 5 is not Associated with Risk of HIV Infection: a Case-control Study

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Abstract

Background—Adenoviruses are among the most promising vectors for the development of an HIV vaccine. The results of the phase IIB study of the adenovirus serotype 5-based Merck Trivalent HIV vaccine have raised the concern that serological immunity to Ad5 could be linked to HIV acquisition risk in high-risk individuals. We examined the association between adenovirus serostatus and the rate of incident HIV infection in populations at elevated risk of HIV acquisition.

Methods—We performed a nested case-control study of Ad5 serostatus among 299 HIV-infected and 590 matched HIV-uninfected persons participating in MACS and in HPTN 039, a study of HSV-2 suppression among adults in the U.S., South America and Africa. Appropriate HIV cases and controls were identified in each cohort, and Ad5 neutralizing antibody titers were compared in these two groups.

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Authors' contributions

MC participated in the design of the study, interpretation of the data and drafted the manuscript. FC participated in the design of the study and drafting of the manuscript. AM performed the statistical analyses and assisted in drafting the manuscript. GS carried out the antibody assays and assisted in drafting the manuscript. AD and JS participated in the design of the study, interpreting the data and drafting the manuscript. CC coordinated study of the HPTN 039 cohort and assisted in interpreting the data and drafting the manuscript. JM and RD coordinated study of the MACS participants and assisted in drafting the manuscript. ME assisted in interpreting the data and drafting the manuscript. LC conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Data

The raw data used in these analyses are available in supplementary table S1

Results—In MACS and HPTN 039, the relative risks of incident HIV infection among Ad5 seropositive vs. Ad5 seronegative individuals were 1.1 (95% CI 0.8–1.5, $p = 0.57$) and 1.0 (95% CI 0.4 – 2.3, $p = 0.99$) respectively. HIV-1 acquisition rates did not vary significantly by Ad5 neutralizing antibody titer.

Conclusions—The presence of Ad5 neutralizing antibodies is not linked to the risk of HIV acquisition among populations at elevated risk of HIV infection.

Keywords

Adenovirus; serology; MSM; HIV; acquisition risk; vaccine

Background

In September of 2007, initial results of the phase IIB efficacy study of the MRK Adenovirus type 5 (Ad5) HIV-1 gag/pol/nef vaccine, known as the Step study [1], suggested that uncircumcised men who entered the trial with serological evidence of prior infection with Ad5 had an increased risk of HIV-1 acquisition after receipt of the vaccine [2]. Enhanced risk of HIV acquisition was not observed among vaccinees who were Ad5 seronegative at enrollment, nor was it associated with vaccine-induced Ad5 neutralizing antibodies, [3–5] which were seen in all vaccine recipients examined. Replication-defective recombinant adenoviral vaccine vectors hold promise for the development of vaccines against several human pathogens including malaria [6], influenza [7], tuberculosis [8], and Ebola virus [9], and vectors based on Ad5, Ad26, and Ad35 are leading candidates in the search for an effective HIV vaccine [10,11]. The completely unexpected trend towards a greater number of HIV infections among Ad5 seropositive vaccinees observed in the Step study therefore raised a serious new concern about the role of pre-existing anti-vector immune responses in modifying the risk of HIV acquisition that has been broadly felt within the vaccine research community [2,12–14].

Natural Ad5 infection is commonly acquired in childhood, and is associated with a mild upper respiratory infection. Seroprevalence to Ad5 varies widely throughout the world, with about 1/3 of those in the US and >80% of those in many areas of South America, Asia and Africa possessing neutralizing antibodies to the virus by mid adulthood [15–17]. The mechanism(s) underlying the apparent enhanced risk of HIV acquisition in Ad5 seropositive vaccinees observed in the Step study are unclear and continue to be debated [5,18,19]. One possible explanation is that past infection with Ad5 may be a biological marker for increased susceptibility to HIV infection. To test this hypothesis, we undertook a retrospective study of Ad5 seroprevalence and HIV incidence in two cohorts: the Multicenter AIDS Cohort Study (MACS), an observational study of U.S. men who have sex with men (MSM), and HPTN 039, a study of HSV-2 suppression among men and women in the U.S., South America and Africa. Selection of these cohorts allowed us to examine the association between Ad5 serology and HIV incidence among a diverse group of MSM and heterosexual women who had a risk of acquiring HIV infection comparable to that of Step study participants.

Methods

Study Design

This study was performed with informed consent and approved by the Ethics Committees overseeing the Centre for Infectious Disease Research in Zambia, the Asociación Civil Impacta Salud y Educación, Lima, Peru and the Multicenter AIDS Cohort Study. To assess the relationship between serological immunity to Ad5 and HIV acquisition risk, we

performed a nested case-control study of Ad5 serostatus among HIV-infected and matched HIV-uninfected persons (cases and controls, respectively). Controls were selected by matching on known risk factors for HIV acquisition in order to isolate the possible effect of Ad5 serostatus on susceptibility and to reduce potential confounding. After identification of cases and controls, we determined Ad5 serostatus (Ad5 neutralizing antibody titer ≤ 18 vs. >18 , the threshold used in post-hoc analyses of the Step study) from stored serum specimens obtained at or near the time of enrollment in the parent study. We then compared the prevalence of Ad5 neutralizing antibodies among cases and controls.

Cohorts

The MACS was established in 1984 as a prospective study of the clinical course of HIV-1 infection in MSM in Baltimore, Chicago, Pittsburgh, and Los Angeles [20]. MACS participants were screened for HIV infection at every 6-month visit. To date, 6,973 men have been enrolled, including 2284 who were HIV-infected at enrollment and approximately 657 who acquired HIV infection after enrollment. HPTN 039 was an NIH-funded Phase III, multi-site, randomized, double-blind, placebo-controlled trial of daily antiviral HSV-2 suppressive therapy for HIV prevention. The study enrolled 3,277 HIV-seronegative, HSV-2 seropositive participants at high risk of HIV infection - women from Zambia, South Africa, and Zimbabwe, and MSM from Peru and the United States. HPTN 039 participants were screened for HIV infection at every 3-month visit. At study termination, 139 incident HIV infections had accrued, including 72 among women and 67 among men [21].

Selection of participants

Cases from the MACS cohort were obtained from the approximately 229 MACS participants who became HIV infected within 2.5 years of enrollment. Controls from this cohort were selected by matching on the following: elapsed time from enrollment in MACS to HIV acquisition (follow-up interval), self-reported lifetime number of anal sex partners, and age at enrollment (within 10 years) (Table 1). Controls were required to have a negative HIV test after an elapsed time equivalent to the follow-up interval for their matched case, and to have at least as many lifetime anal receptive sex partners as the case. When appropriate controls meeting this degree of stringency were not available, cases were matched to controls with up to 2 fewer lifetime partners. Circumcision status was not available on all study subjects, and therefore, matching was not performed on circumcision status. However, this factor was evaluated as a potential confounding factor (see results).

HPTN 039 cases were participants who acquired HIV infection between study enrollment and study completion at 18 months. Controls from this study were selected by matching on the following: country of residence, time from enrollment to HIV acquisition, gender, self-reported circumcision status (males), and self-reported number of sexual partners in the previous month (males). Since 92% of women had only one lifetime partner and 99.9% had no more than 2 partners, the number of partners in the previous month was not used as a matching criterion for women.

Serological Assay for adenovirus immunity

Ad5 neutralizing antibody titers were determined by the HIV Vaccine Trials Network (HVTN) laboratory (FHCRC, Seattle, WA) using the methodology employed in the Step study [22]. Neutralization activity was measured in blinded clinical samples using a recombinant Ad5 carrying a reporter gene for secreted alkaline phosphatase (SEAP). Equal volumes of virus and test serum were combined at serum dilutions ranging from 1:18 to 1:4608, plated alongside negative and positive serum controls, and incubated for one hour. Neutralization reactions were added to HEK 293 cells seeded at 3×10^4 cells/well in 96-well plates. After one hour, the infection mix was removed and the wells were re-fed with

complete medium. One day post infection, 50 μ l of media was used to determine the concentration of SEAP by measuring the chemiluminescent substrate (CSPD®). The neutralization titer was defined as the serum dilution giving a 50% reduction of SEAP activity relative to SEAP activity from virus infection alone. Titers ≤ 18 were considered negative. Sera from enrollment visits were tested in each cohort.

Power Analyses

Because Ad5 seroprevalence varies geographically, we used Ad5 seroprevalence rates from several regions represented in the Step study to estimate the sample size needed for a sufficiently powered analysis. In the Step study Ad5 seroprevalence was 43% in the United States (males), 49% in Canada (males), and 66% to 89% among males and 73% to 100% among females residing in Brazil, the Dominican Republic, Haiti, Jamaica, Peru and Puerto Rico. Based on assumed seroprevalence rates of 40–50% in the MACS cohort and 70–90% in HPTN 039, we determined that 214 MACS cases and 139 HPTN cases (and 2 controls per case) would yield at least 80% power to detect a 20% increase in the relative risk of Ad5 seropositivity associated with case status, corresponding to an absolute increase in Ad5 seropositivity of 8–12%. No substantial increase in power was obtained for either cohort by increasing the control to case ratio to three, and therefore two controls were used for each case.

Statistical Analysis

The rates of Ad5 seropositivity in cases and controls were compared, within each cohort. Conditional logistic regression was performed to obtain the unadjusted odds ratio for HIV infection by Ad5 seropositivity (defined as an Ad5 Nab titer > 18). Since cases and controls were matched for known risk factors, adjusted analyses including these matching factors were not performed. For each cohort, the ratios of observed to expected cases within strata of Ad5 NAb titer (0.25 log increments) were calculated; observed to expected case ratios were plotted against Ad5 NAb titer to examine trends. Additional univariate and multivariate conditional logistic regressions were performed including self-reported circumcision status and CCR5 Δ 32 genotype in MACS participants. All analyses evaluating the relationship between Ad seroprevalence and HIV acquisition were performed at the University of Washington.

Results

Cases and controls in both cohorts were demographically similar (Table 1). MACS cohort: we obtained baseline Ad5 titers on 214 cases and 423 matched controls who did not become infected during an equivalent observation period. Ad5 titers > 18 were found in 246 persons near the time of acquisition or censoring. The rates of Ad5 seropositivity differed by no more than 2% between cases and controls (Table 1). The odds ratio for incident HIV infection among Ad5-seropositives vs. Ad5-seronegatives was 1.1, which was not statistically significant (95% CI = 0.8 – 1.5, $p = 0.57$).

Because participants from the MACS cohort were not matched on circumcision status, a factor associated with HIV acquisition in Step study vaccinees, we sought to evaluate the effect of circumcision on the relationship between Ad5 serostatus and risk of HIV infection. Circumcision status was available for 106 cases and 317 controls. Eighty-eight % (93/106) of cases and 97% (306/317) of controls were circumcised ($p = 0.001$). Circumcision status was not associated with Ad5 serostatus; 42% (10/24) of uncircumcised men had detectable Ad5 nAb titers, versus 37% (148/399) of circumcised men ($p = 0.63$). These observations indicate that the lack of association between Ad5 and HIV acquisition is unlikely to be attributable to confounding or competing effects of circumcision and Ad5 exposure.

CCR5Δ32 deletion genotype information was available on 359 persons; 64 individuals were heterozygous for CCR5Δ32, including 41 Ad5 seronegatives and 23 Ad5 seropositives. 8 individuals were CCR5Δ32 homozygous, including 3 Ad5 seronegatives and 5 Ad5 seropositives. There was no significant association between Ad5 serostatus and presence of the CCR5Δ32 deletion, in either the heterozygous or homozygous state ($RR = 0.87$, $p = 0.48$ and $RR = 1.52$, $p = 0.14$, respectively). In separate adjusted conditional logistic regressions, including a) circumcision status and b) presence of either one or two copies of the CCR5Δ32 deletion, the odds of being a case in the MACS cohort were not associated with Ad5 detection ($p = 0.14$ and $p = 0.45$, respectively). When these analyses were repeated while excluding the 8 individuals homozygous for the CCR5Δ32 deletion, again there was no association between HIV-1 case status and presence or absence of Ad5 neutralizing antibody titers > 18 ($p = 0.45$).

HPTN 039 cohort: Of 254 original participants, 252 participants were included in analysis. Two controls were excluded as there was not a valid titer for Ad5 in the corresponding case, and three cases only had one control with valid Ad5 serostatus. Thus the analysis was performed with 85 cases and 167 controls (Table 1). The 31 cases and matching controls from Zambia were women while the remaining participants were men. In this cohort, the odds ratio for incident HIV infection among Ad5-seropositives vs. Ad5-seronegatives was 1.0 (95% CI = 0.4 – 2.3, $p = 0.99$). Inclusion of circumcision status in the regression model for HPTN039 also did not alter the significance of Ad5 detection versus the case status (OR = 1.0, 95% CI = 0.4 – 2.5, $p = 0.99$). Information on CCR5Δ32 genotype was not available in this cohort. In neither MACS nor HPTN039 was there an apparent relationship between Ad5 titer and HIV acquisition rate (Figure 1).

Discussion

An apparent association between Ad5 seropositivity and increased acquisition of HIV in the Step trial has raised the concern that Ad5 serological immunity could be a marker of enhanced susceptibility to HIV infection. Here, we present a nested case-control study of Ad5 serostatus and HIV acquisition risk in men and women at elevated risk of HIV infection, adjusted for potential confounders including geographic region, circumcision status (among MSM), number of sexual partners and CCR5Δ32 deletion genotype. Our study evaluates a significantly greater number of HIV acquisition cases than occurred in the Step study (299 cases vs. 84 cases at termination of Step study), and therefore provides a more powerful statistical analysis of the association between Ad5 seropositivity and HIV acquisition in the absence of vaccination.

We found no association between Ad5 neutralizing antibody seropositivity and incidence of HIV infection, and this lack of association remained unaffected by circumcision status and CCR5Δ32 genotype among those in whom this information was available. In addition, no clear relationship between Ad5 titer and HIV acquisition rate was evident (Figure 1), although this study was not specifically designed to stratify HIV acquisition risk by Ad5 titer. These results demonstrate that in two cohorts at elevated risk of HIV infection similar to that recruited to the Step study, Ad5 seropositive individuals were no more likely than Ad5 seronegative individuals to acquire HIV. Therefore, past Adenovirus type 5 infection, as measured by presence of Ad5 neutralizing antibodies, does not appear to be a marker of increased susceptibility to HIV infection in unvaccinated individuals.

In Step, the trend towards greater risk of HIV infection among individuals with Ad5 neutralizing antibodies prior to vaccination did not reach statistical significance, and further analyses revealed that HIV acquisition among Step study participants who received the Ad5 vectored vaccine was more highly associated with lack of circumcision than with baseline

Ad5 serostatus [23,24]. We note that the Step study was not powered to perform subgroup analyses, especially between circumcised and uncircumcised men. The present study does not exclude the possibility of an interaction between pre-existing serological immunity to Ad5 and vaccination with Ad5-vectored vaccines in modifying the risk of HIV acquisition, which must be addressed in separate studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Ad5 neutralizing antibody titers and HIV acquisition

Normalized case rates by Ad5 titer. Ratio of observed to expected case rate (y-axis) by Ad5 titer (x-axis). Since two controls were selected per case, the expected case rate assuming that Ad5 serostatus has no effect on the risk of HIV acquisition is 33%, and the expected ratio is 1. Ad5 seronegative participants are those with Ad5NAb <18. The number of subjects in each antibody titer stratum is indicated above the corresponding bar.

Table 1**Cohort Demographics**

Demographic information on cases and controls, by cohort.

	HPTN039 study		MACS cohort	
	Controls (N=167)	Cases (N=85)	Controls (N=423)	Cases (N=214)
Enrollment characteristics				
Male gender	107 (64%)	54 (64%)	423 (100%)	214 (100%)
Age (median (IQR)) [†]	30 (25, 39)	25 (22, 30)	30 (26, 36)	31 (27, 36)
Lifetime sex partners [*] (median (IQR))			6 (3, 15)	6 (4, 15)
Partners in previous month (males)				
0	12 (11%)	6 (11%)		
1	17 (16%)	9 (17%)		
2–3	42 (39%)	21 (39%)		
4+	36 (34%)	18 (33%)		
Circumcised (males) [‡]	8 (8%)	4 (7%)	306 (97%)	93 (88%)
Site				
Lima, Peru	101 (60%)	51 (60%)		
San Francisco, USA	2 (1%)	1 (1%)		
Seattle, USA	4 (2%)	2 (2%)		
Lusaka, Zambia	60 (36%)	31 (36%)		
Site (USA) [‡]				
Baltimore, MD			148 (35%)	57 (27%)
Chicago, IL			90 (21%)	49 (23%)
Pittsburgh, PA			110 (26%)	49 (23%)
Los Angeles, CA			75 (18%)	59 (28%)
Ad5 serostatus by subgroup				
Ad5 detection	147 (87%)	75 (88%)	160 (38%)	86 (40%)
OR (95%CI), p-value	1.0 (0.4–2.3) p = 0.99	1.1 (0.8–1.5)	p = 0.57	

* Anal receptive sex partners only.

[†] Matching was not required by age since participants are matched on time of followup, however it was performed for participants of MACS cohort.

[‡] Matching was not initially done on site or circumcision status for MACS cohort.