Extended Follow-up Confirms Early Vaccine-Enhanced Risk of HIV Acquisition and Demonstrates Waning Effect Over Time Among Participants in a Randomized Trial of Recombinant Adenovirus HIV Vaccine (Step Study)

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Background. The Step Study tested whether an adenovirus serotype 5 (Ad5)–vectored human immunodeficiency virus (HIV) vaccine could prevent HIV acquisition and/or reduce viral load set-point after infection. At the first interim analysis, nonefficacy criteria were met. Vaccinations were halted; participants were unblinded. In post hoc analyses, more HIV infections occurred in vaccinees vs placebo recipients in men who had Ad5-neutralizing antibodies and/or were uncircumcised. Follow-up was extended to assess relative risk of HIV acquisition in vaccinees vs placebo recipients over time.

Methods. We used Cox proportional hazard models for analyses of vaccine effect on HIV acquisition and vaccine effect modifiers, and nonparametric and semiparametric methods for analysis of constancy of relative risk over time.

Results. One hundred seventy-two of 1836 men were infected. The adjusted vaccinees vs placebo recipients hazard ratio (HR) for all follow-up time was 1.40 (95% confidence interval [CI], 1.03–1.92; P = .03). Vaccine effect differed by baseline Ad5 or circumcision status during first 18 months, but neither was significant for all follow-up time. The HR among uncircumcised and/or Ad5-seropositive men waned with time since vaccination. No significant vaccine-associated risk was seen among circumcised, Ad5-negative men (HR, 0.97; P = 1.0) over all follow-up time.

Conclusions. The vaccine-associated risk seen in interim analysis was confirmed but waned with time from vaccination.

Clinical Trials Registration. NCT00095576.

Despite the recent success of human immunodeficiency virus (HIV) prevention interventions such as male circumcision, pre-exposure prophylaxis, and microbicides, an effective prophylactic HIV vaccine remains the best long-term strategy for preventing HIV infection and AIDS. Several lines of evidence suggested the importance of HIV type 1 (HIV-1)–specific T cells in reducing progression and even acquisition of infection. The Step Study, a phase 2B trial, tested the ability of
an HIV vaccine using a replication-defective adenovirus serotype 5 (Ad5) vector with subtype B HIV-1 gag/pol/nef inserts to prevent HIV infection and/or to reduce early HIV viral load among study participants who became infected after vaccination [1]. This vaccine had been shown to provide amelioration of disease course in nonhuman primates after challenge with HIV-SIV hybrid virus (SHIV) [2, 3] and elicited high-level T-cell responses in humans. Three thousand persons, stratified on their preexisting antibody titers to Ad5, were enrolled between December 2004 and March 2007. The initial planned interim analysis in September 2007 crossed prespecified futility boundaries for efficacy, indicating no significant reduction in HIV acquisition or early viral load in participants with baseline Ad5-neutralizing antibody titers of <200. Vaccinations were halted, and participants were unblinded. In post hoc analyses of data collected before unblinding, more HIV infections were seen among vaccinees than among placebo recipients in some subgroups, notably men who were uncircumcised or who had neutralizing antibodies to Ad5 (Ad5 seropositive) at enrollment.

To assess the longer-term risk of HIV infection among Step Study participants, the HIV Vaccine Trials Network (HVTN) collaborated with Merck to extend follow-up of Step Study participants. After unblinding, participants were encouraged to remain in follow-up for up to 4 years from the time of first vaccination or until 31 December 2009, whichever came first. We report here on the risk of HIV acquisition among vaccinees vs placebo recipients, overall and in the subgroups of male participants stratified by baseline circumcision and Ad5 serostatus, during the entire follow-up period. We also assessed whether vaccine effect and the impact of potential vaccine effect modifiers changed over time.

METHODS

Study Design and Population
The Step Study was conducted in regions of the world where clade B is the predominant HIV-1 subtype (North America, the Caribbean, South America, and Australia), and the study included high-risk, uninfected men who have sex with men and high-risk heterosexual men and women [1]. Participants received a vaccine or placebo at baseline, 4 weeks, and 26 weeks. Randomization was prestratified by study site, gender, and baseline Ad5 titer (<18, 18–200, 201–1000, >1000). In 2007, after participants were unblinded, they were encouraged to continue to be followed in the Step study and subsequently in a roll-over protocol, HVTN 504.

Procedures
The Step Study procedures have been previously described [1]. Briefly, participants were tested for HIV at screening, enrollment, 12 weeks, and 30 weeks and then every 6 months thereafter in the Step Study, which was anticipated to last 4 years. After the interim analysis and unblinding, participants were encouraged to continue follow-up in the Step Study and to enroll in HVTN 504 when it opened at their site. Those who consented could join HVTN 504 at any point during their follow-up; after entering HVTN 504, HIV-uninfected participants had study visits every 3 months until they had completed a combined follow-up totaling 4 years or until 31 December 2009, whichever came first. Study procedures and data collected in the 2 studies were identical, except that HVTN 504 contained additional behavioral assessments at 3-month intervals. Screening for HIV infections among study participants was conducted in a central laboratory and used serologic tests (enzyme immunoassay) (Uni-Gold Recombigen HIV Test; Trinity BioTech; or Multispot HIV-1/HIV-2 Rapid Test, Bio-Rad) that detected HIV components that were not in the vaccine. All infections were confirmed by Western blot and/or viral-load testing and verified by an independent, blinded adjudication committee. Specimens taken prior to the visit when HIV was first detected serologically were tested for HIV RNA (Amplicor Monitor version 1.5; Roche) to establish the earliest time of detectable HIV infection. All participants with HIV infection were followed with viral-load and CD4+ T-cell count monitoring on a separate schedule, which was identical for the Step and HVTN 504 studies [4]; participants were referred for antiretroviral treatment according to local treatment guidelines. Institutional review board approval for the Step Study and for HVTN 504 was obtained at all study sites, and all participants gave written informed consent.

Study Objectives and Statistical Analysis
All analyses described below assess the modified intent-to-treat (MITT) male cohort—that is, all men who were HIV negative at study entry and who received at least 1 vaccination. Follow-up time from enrollment in the Step Study until the end of follow-up (in the Step Study or HVTN 504) (see Figure 1) was included for analysis.

Vaccine Effect on HIV Infection
The assumption typically made for randomized, double-blind clinical trials that risk exposure in the treatment groups is equal may be less warranted here due to early unblinding of treatment assignment and longer follow-up. To address this, we adopted 2 modeling strategies to estimate the effect of treatment on HIV incidence, namely a multivariate Cox proportional hazards model that adjusts for baseline confounders and a semiparametric method that estimates the unconditional hazard ratio (HR) using the baseline covariates as auxiliary variables [5]. Because the results are similar, only those from the former were reported. We also displayed the treatment effect over time by curves of the cumulative probability of HIV infection in vaccine or placebo recipients, adjusted for
baseline covariates via the Cox model, as well as the difference between the two curves.

Based on all-subsets model selection using the exact Akaike’s information criterion, we selected the following potential baseline confounding variables for inclusion in the Cox models using all available data: age (≤30 years vs >30 years), herpes simplex virus type 2 seropositivity (yes or no), race (white or other), region (North America + Australia vs other), any drug use (yes or no), number of male partners (≥4 vs <4), unprotected insertive anal sex (yes or no), and unprotected receptive anal sex (yes or no).

The time-to-event variable in the survival analysis was defined as time from first vaccination to estimated time of infection. For most HIV-infected participants, time to infection was defined as the time from initial vaccination to the midpoint between the date of the last visit with no evidence of HIV infection (HIV seronegative and HIV-1 plasma viral RNA negative) and the date of the first serologic evidence of HIV-1 infection. We also estimated the time of infection using the Fiebig stages, which are average values for time from infection to the development of detectable HIV RNA, p24, and HIV antibodies [6]. For HIV-infected subjects whose first positive specimen was HIV antibody negative but HIV-1 plasma viral RNA positive, the estimated time of HIV infection was defined as 16 days prior to specimen collection to account for the duration of the eclipse period and early stages of HIV infection prior to seroconversion (Fiebig stages 1 and 2). For subjects who never showed any evidence of HIV-1 infection, their time-to-event variable was right censored on the date of their last blood draw.

**Covariate Effect and Vaccine-Induced HIV Infection Risk**

We used interaction tests based on Cox models to assess whether baseline Ad5 serostatus and circumcision status act as potential vaccine effect modifiers. We also employed a potential outcome-based framework to evaluate the impact of quantitative baseline Ad5 titers on the probability that a vaccine recipient would be infected if assigned the vaccine but not if assigned the placebo (ie, the probability of increased infection risk associated with vaccination). We estimated this probability as a function of baseline Ad5 titer under the assumption that no vaccine recipient was protected from infection [7]. We continued to investigate vaccine effect on HIV infection in the following 4 subgroups of men: uncircumcised, Ad5-seropositive men; uncircumcised, Ad5-seronegative men; circumcised, Ad5-seropositive men; and circumcised,
Ad5-seronegative men. To account for multiple hypothesis tests associated with these mutually exclusive subgroups, \( P \) values for the significance of the HR estimates from the subgroup-specific Cox models were adjusted by the Bonferroni method. Because increased risk of HIV infection associated with vaccination is of more concern, we provided 1-sided \( P \) values in all subgroup analyses. We provided 2-sided \( P \) values for other analyses. A type 1 error rate of 0.05 was used in all analyses.

**Time-Varying Vaccine Effect on HIV Infection**

To assess possible changes of relative risk of vaccinees vs placebo recipients with time since vaccination, a time-dependent covariate indicating a breakpoint at 18 months postenrollment (ie, 12 months after the last vaccination) was included in Cox models that test for the interaction between treatment and the 18-month breakpoint. The 18-month cutoff was chosen for biologic reasons, because this represents data accumulated within 1 year of the last vaccination when vaccine-induced effects are expected to be highest, and for operational reasons, because the number of infections and person-years (PYs) of follow-up occurring within 18 months of enrollment and after 18 months were roughly equal (Table 1). Graphically, instantaneous HR during the entire study period with 95% simultaneous confidence intervals (CIs) were used to display the treatment effect on HIV acquisition over time [8]. Grambsch and Therneau’s [9] test was applied to assess whether the HR was constant over time.

**Risk Behavior, Loss to Follow-up, and Sensitivity Analysis to Assess Robustness of Observed Results**

Chi-squared tests were used to assess possible differences in risk behaviors at baseline and at later time points between treatment arms. Cox models were used to assess predictors of loss to follow-up among uninfected subjects. Variables examined include all the aforementioned baseline characteristics and treatment assignment. Chi-squared tests were used to assess association between treatment and baseline variables among uninfected subjects who terminated early.

Multivariate models were used to take into account potential confounding of measured prognostic factors. However, uncounted imbalances between treatment arms may still bias the association under study, especially for observations in the >18 month analysis period because most infections (78 of 83 [94%]) here occurred after unblinding. Sensitivity analyses were therefore performed to examine, both independently and jointly, the impact of unmeasured prognostic factors of HIV infection and the impact of potential differential drop-out on HR estimates. We have looked specifically at potential imbalance of participants’ characteristics that could develop after unblinding. More details are described in the Supplementary materials.

**RESULTS**

**Descriptive Results**

Among the 3000 male and female Step Study participants, 2060 enrolled in HVTN 504 (Figure 1). The demographics of the MITT cohort have been reported previously [1]. Because few (2 prior to unblinding and 13 post-unblinding) HIV infections were detected among women, with HIV incidence of 0.45 per 100 PYs (95% CI, .25–.74), we have limited all subsequent analyses to men. The majority (1704 of 1836 [93%]) of male participants received all 3 vaccinations, 96 (5%) received

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>HIV Incidence per 100 Person-Years (95% CI)</th>
<th>No. of MITT Males at Risk</th>
<th>HIV Infections</th>
<th>Person-Years of Follow-up</th>
<th>HIV Infections</th>
<th>Person-Years of Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncircumcised and Ad5-seropositive men</td>
<td>2.54 (1.86–3.39)</td>
<td>622</td>
<td>22</td>
<td>850.9</td>
<td>24</td>
<td>958.2</td>
</tr>
<tr>
<td>Uncircumcised and Ad5-seronegative men</td>
<td>3.78 (2.28–5.91)</td>
<td>171</td>
<td>12</td>
<td>237.3</td>
<td>7</td>
<td>264.4</td>
</tr>
<tr>
<td>Circumcised and Ad5-seropositive men</td>
<td>4.04 (2.96–5.38)</td>
<td>419</td>
<td>24</td>
<td>557.0</td>
<td>22</td>
<td>579.5</td>
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<tr>
<td>Circumcised and Ad5-seronegative men</td>
<td>3.42 (2.60–4.41)</td>
<td>578</td>
<td>28</td>
<td>792.2</td>
<td>31</td>
<td>929.1</td>
</tr>
<tr>
<td>Totala</td>
<td>3.29 (2.81–3.82)</td>
<td>1790</td>
<td>86</td>
<td>2437.4</td>
<td>84</td>
<td>2731.2</td>
</tr>
<tr>
<td>Totaleb</td>
<td>3.30 (2.83–3.83)</td>
<td>1836</td>
<td>88</td>
<td>2452.4</td>
<td>84</td>
<td>2744.7</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

* Excludes 46 participants who were missing circumcision status.

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2 vaccinations, and 36 (2%) received 1 vaccination. The median total follow-up time was 3.23 (range, 0–4.20) years.

There were 172 infections among 1836 men during the entire follow-up period: 88 before 18 months and 84 afterwards. The HIV incidence was 3.30 per 100 PYs (95% CI, 2.83–3.83) over all follow-up time, 3.59 per 100 PYs (95% CI, 2.88–4.42) within 18 months of enrollment, and 3.06 per 100 PYs (95% CI, 2.44–3.79) thereafter. The HIV incidence rates in the 4 subgroups defined by baseline circumcision status and adenovirus serotype 5 (Ad5) antibody serostatus: uncircumcised, Ad5-seropositive men (Uncirc/Ad5+) (A); uncircumcised, Ad5-seronegative men (Uncirc/Ad5−) (B); circumcised, Ad5-seropositive men (Circ/Ad5+) (C); and circumcised, Ad5-seronegative men (Circ/Ad5−) (D).

**Figure 2.** Overall and subgroup cumulative incidence of human immunodeficiency virus (HIV) infections over 48 months of follow-up. Covariate-adjusted probability of HIV infection curves and their differences (placebo group − vaccine group [P − V]) are shown for vaccine and placebo recipients overall and by baseline circumcision status and adenovirus serotype 5 (Ad5) antibody serostatus: uncircumcised, Ad5-seropositive men (Uncirc/Ad5+) (A); uncircumcised, Ad5-seronegative men (Uncirc/Ad5−) (B); circumcised, Ad5-seropositive men (Circ/Ad5+) (C); and circumcised, Ad5-seronegative men (Circ/Ad5−) (D).

Inferential Analysis Results

**Vaccine Effect on HIV Infection**
Over all follow-up time, there was a higher risk of HIV infection among the vaccine recipients than among placebo recipients (Figure 2, upper panel). The covariate-adjusted HR was 1.44 (95% CI, 1.05–1.97; P = .03).
Table 2. Covariate-Adjusted Hazard Ratios (vaccine:placebo) for All Incident HIV Infections That Occurred During the First 18 Months After Enrollment and During All Follow-up Time

<table>
<thead>
<tr>
<th>Subgroupa</th>
<th>First 18 Months of Follow-up</th>
<th></th>
<th></th>
<th>Follow-up From 18 Months After Enrollment to End of Study</th>
<th></th>
<th></th>
<th>All Follow-up</th>
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<tbody>
<tr>
<td></td>
<td>Hazard Ratiob (95% CI)</td>
<td>Multiple Comparison—Adjusted P Valuec</td>
<td>Hazard Ratiob (95% CI)</td>
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<td>Hazard Ratiob (95% CI)</td>
<td>Multiple Comparison—Adjusted P Valuec</td>
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<td></td>
</tr>
<tr>
<td>Uncircumcised and Ad5-seropositive men</td>
<td>4.18 (1.37–12.71)</td>
<td>.02</td>
<td>0.69 (.29–1.62)</td>
<td>.79d</td>
<td>1.58 (.86–2.93)</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncircumcised and Ad5-seronegative men</td>
<td>2.66 (.65–10.86)</td>
<td>.34</td>
<td>1.24 (.27–5.76)</td>
<td>1.0</td>
<td>2.35 (.86–6.39)</td>
<td>.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumcised and Ad5-seropositive men</td>
<td>1.98 (.84–4.67)</td>
<td>.24</td>
<td>1.33 (.55–3.17)</td>
<td>1.0</td>
<td>1.61 (.88–2.94)</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumcised and Ad5-seronegative men</td>
<td>0.38 (.16–90)</td>
<td>.06d</td>
<td>2.18 (.97–4.92)</td>
<td>.12</td>
<td>0.97 (.56–1.65)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.48 (.96–2.28)</td>
<td>1.13 (.86–2.09)</td>
<td>1.40 (1.03–1.92)</td>
<td></td>
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Abbreviations: Ad5, adenovirus serotype 5; CI, confidence interval; HIV, human immunodeficiency virus.

a Data on 46 of 1836 men not included due to missing data on circumcision status.

b All models were adjusted for the same baseline covariates: age (≤30 years vs >30 years), herpes simplex virus type 2 seropositivity (yes or no), race (white or otherwise), region (North America, Australia, or otherwise), any drug use (yes or no), number of male partners (≥4 vs <4), unprotected insertive anal sex (yes or no), and unprotected receptive anal sex (yes or no).

c Bonferroni procedure-adjusted P values corrected for multiple subgroup comparisons.

d One-sided P values for testing hazard ratio < 1.

Covariate Effect and Vaccine-Associated HIV Infection Risk

During the first 18 months, there was a significant 3-way interaction between treatment, Ad5 serostatus, and circumcision status (P = .04), suggesting a joint effect of the 2 baseline factors on the relative risk of HIV infection. In subgroup analyses, HR estimates in the 3 subgroups with either or both of these baseline factors were >1. The greatest HR was seen among uncircumcised, Ad5-seropositive men; in this group, the risk among vaccine recipients was >4 times that seen in placebo recipients and remained significant even after adjustment for other baseline covariates (Table 2). The adjusted 1-sided P values indicated that in the other 3 subgroups risk of infection in vaccinees was not statistically significantly higher than risk in placebo recipients.

We observed that prior to unblinding the difference in risk of HIV infection attributable to vaccination increased as a function of the baseline Ad5 titer. The vaccine-attributable increase in risk was 1%–4%; the difference in risk for HIV infection increased with higher baseline Ad5 titer (Figure 3). However, the lower 95% CIs of the risk difference are near 0 (Figure 3). In analyses that included all pre- and post-unblinding data, this pattern of increasing difference in risk as a function of baseline Ad5 titer holds up after adjustment for other baseline covariates (data not shown). Over all follow-up time, more incident HIV infections occurred among vaccine recipients than among placebo recipients in the 3 subgroups with Ad5-seropositive and/or uncircumcised men (Figure 2, lower panel). However, none of the HRs was significantly >1 or <1; adjusted P values were nonsignificant (Table 2). Furthermore, the impact of baseline Ad5 serostatus (interaction test, P = .2) or circumcision status (P = .4) on the vaccine effect was not evident over the entire follow-up period.

Vaccine Effect on HIV Infection Over Time

In the entire MITT male cohort, there was no significant change in the vaccinees vs placebo recipients HR over time (P = .92). The vaccinees vs placebo recipients HR was 1.48 during the first 18 months and 1.34 thereafter (Table 2). However, significant changes in the HRs were seen for 2 subgroups even after adjustment of multiplicity (Table 2): uncircumcised, Ad5-seropositive men (interaction test, P = .04), and circumcised, Ad5-seronegative men (P = .04). Among uncircumcised, Ad5-seropositive men, the initially high HR of 4.18 (95% CI, 1.37–12.71; P = .02) in the first 18 months decreased to 0.69 (95% CI, 0.29–1.62; P = .79) thereafter. Although the initial HR of 0.38 (95% CI, 0.16–90; P = .06) among circumcised, Ad5-seronegative men increased after 18 months to 2.18 (95% CI, 0.97–4.92; P = .12), the second value did not represent a statistically significant elevation in risk among vaccine recipients.

Among uncircumcised and/or Ad5-seropositive men (combining the 3 subgroups with HR estimates ≥1), the elevated risk of infection in the vaccine group during the first 18 months appeared to wane over time (unadjusted P = .06 for ≤18 months vs >18 months). The elevation of risk was not
Risk Behavior, Loss to Follow-up, and Sensitivity Analysis to Assess Robustness of Observed Results

In the 4 subgroups analyzed, self-reported risk behaviors did not differ significantly in vaccine vs placebo recipients at baseline or over time. No difference was seen in the proportion of vaccinees vs placebo recipients who reported unprotected receptive anal intercourse at 24 or 36 months in any of the 4 subgroups examined (uncircumcised, Ad5-seropositive: adjusted $P = .63$ and $.65$ at 24 and 36 months, respectively; uncircumcised Ad5-seronegative: $P = 1.0$ and $1.0$; circumcised, Ad5-seropositive: $P = 1.0$ and $1.0$; circumcised, Ad5-seronegative: $P = 1.0$ and $1.0$). Data for drug use, number of sexual partners, and unprotected insertive anal intercourse were similar. In addition, baseline variables relating to sexual risk: number of male partners ($P = .97$) and unprotected receptive ($P = .54$) or unprotected insertive anal intercourse ($P = .41$) were not associated with loss to follow-up among uninfected men. There was no evidence that relative loss to follow-up in vaccine vs placebo recipients differed before and after 18 months postenrollment ($P = .44$).

Among uninfected men, higher loss to follow-up was associated with receiving placebo ($P = .03$), age <30 years ($P = .02$), residing in North America ($P = .004$), and use of speed ($P = .02$), poppers ($P = .01$), or any drug ($P = .04$). Neither circumcision nor baseline Ad5 status was associated with loss to follow-up. Among those lost to follow-up, no imbalance between the treatment groups was observed for any of the baseline variables (data not shown).

Sensitivity analysis was conducted to assess the potential effect of unmeasured prognostic factors and differential loss to follow-up, independently and jointly, on the HR estimates (Supplemental Table 1A and 1B). Under the assumptions outlined in these analyses, described in detail in the Supplementary materials, relatively large imbalances in unmeasured prognostic factors (1.6–4.0 fold) or differential drop-out (1.2–3.0 fold) in vaccinees vs placebo recipients would be needed to explain either the observed overall HR or the waning vaccine effect had there been no vaccine effect or had the true vaccine effect been constant over time.

DISCUSSION

These results extend the findings of the interim analysis of the Step Study data. The combined data set showed an increased risk of HIV infection associated with vaccination among the entire study population (adjusted HR, 1.44; 95% CI, 1.05–1.97). The difference in risk of HIV acquisition prior to unblinding increased with baseline Ad5 titer, a dose-effect relationship that suggests but does not prove a biologic effect of prior Ad5 immunity on susceptibility to HIV infection after vaccination. Vaccine-associated risk appeared to be highest shortly after vaccination and to decrease after 18 months for uncircumcised and/or Ad5-seropositive men, especially the subgroup of uncircumcised, Ad5-seropositive men in whom the hazard was highest initially. The HR was never elevated in circumcised, Ad5-seronegative men.

Enhanced susceptibility to HIV infection has not been reported in other clinical trials of HIV vaccines [1 10–14].
Altered disease severity or presentation has been reported previously for other vaccines, such as respiratory syncytial virus vaccine [15] or inactivated measles virus vaccine [16]. Antibody- and complement-dependent enhancement of HIV infection has been described in vitro associated with antibodies to gp120 [17, 18], and enhanced simian immunodeficiency virus (SIV) replication has been reported in nonhuman primates after vaccination with an SIV envelope protein [19]. This mechanism seems unlikely to play a role here because the Step Study vaccine did not contain env antigens.

Our findings are compatible with biologic changes, such as increased numbers of HIV target cells at mucosal sites due to vector-specific anamnestic responses shortly after vaccination, or post-unblinding events, such as differential loss to follow-up and imbalances in unmeasured prognostic factors. The unblinding date and 18-month cut-offs created similar groups; 83 of 88 (94%) infections prior to 18 months occurred before unblinding, and 79 of 84 (94%) infections after 18 months occurred after the unblinding date. Thus, it is unclear whether the decreasing relative risk seen in uncircumcised and/or Ad5-seropositive men is due to waning vaccine effect, a regression effect, differential loss to follow-up, or differences in behavior change in vaccinees vs placebo recipients. Although we cannot rule out such changes, our sensitivity analysis indicates that imbalances between treatment groups would need to be large to explain the observed results. In addition, we saw no differences by treatment arm in predictors of loss to follow-up or in ongoing risk behavior among study participants. Vaccinees and placebo recipients were well matched, but, because enrollment was not balanced across sites with respect to Ad5 and circumcision status, men in the 4 groups differed by factors such as race, region, and self-reported risk behavior. Thus it is difficult to correct for possible differences between circumcised and uncircumcised men or Ad5-seropositive and Ad5-seronegative men, who likely differed in important ways other than circumcision or Ad5 status. Given our results, it seems prudent to avoid use of Ad5-vectored vaccines in populations of uncircumcised or Ad5-seropositive men until more data become available. In contrast, a population (circumcised, Ad5-seronegative men) in which vaccination did not carry an increased risk of HIV infection was clearly identified.

These limitations notwithstanding, the study had many strengths. The standardized study procedures and definitions used in the Step Study and HVTN 504 allowed the construction of a combined data set. The more complete follow-up allowed us to look at subgroups with more precision. Finally, multiple models and sensitivity analyses were conducted to control for potential effects of differential loss to follow-up as well as measured and unmeasured confounders.

In 2009, the RV144 Trial demonstrated that at least some protection against HIV-1 infection can be achieved by a vaccine; the regimen used contained HIV envelope as well as gag and pol [10]. Interestingly, the CD8 T-cell epitope responses to the MRK Ad5 gag/pol/nef vaccine used in the Step Study were frequent and strong, whereas participants in the RV144 Trial had rarer CD8 responses but were more likely to have CD4 responses. Although ongoing work has uncovered some potential correlates of risk of infection in the RV144 Trial [20], our understanding of the correlates of vaccine protection against HIV infection remains far from complete. Ongoing research using specimens from Step and RV144 study participants should inform future trials by delineating vaccine-induced responses that are irrelevant or harmful from those that protect.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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