HIV Seroconversion without Infection after Receipt of Adenovirus-Vectored HIV Type 1 Vaccine

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Background. We analyzed human immunodeficiency virus (HIV) seroresponses from 3 phase I HIV-1 vaccine trials to assess the frequency of vaccine-induced seroconversion.

Methods. HIV-1 and HIV-2 enzyme-linked immunosorbent assay (ELISA) was performed during trials of adenovirus type 5 (Ad5)–vectored clade B HIV-1 monovalent gag and trivalent gag/pol/nef vaccines given to HIV-seronegative adults. Doses were administered at day 1, week 4, and week 26. Results were analyzed by vaccine formulation and dose and were stratified by baseline Ad5 titer. ELISA-positive samples were reflexively tested by Western blotting.

Results. Overall, 165 (41%) of 406 evaluable vaccine recipients had positive ELISA results but negative PCR results by week 78. Seroconversion rates were directly related to vaccine dose, were inversely related to baseline Ad5 titer, and were unaffected by vaccine valency. One hundred (89%) of 113 evaluable patients with low baseline Ad5 antibody titers (≤ 200) who were given 1 dose of vaccine with ≥ 1 × 10^10 gag-containing Ad5 particles per dose experienced seroconversion. Of 163 vaccine recipients who had positive ELISA results and available Western blot results, 150 (92%) had indeterminate results of Western blot, typically involving bands at p24, p40, and/or p55. Thirteen uninfected patients (8%) had equivocally positive Western blot results, usually because of an additional weak glycoprotein 41 band. Env-specific enzyme immunoassay results were falsely positive for 2 uninfected vaccine recipients.

Conclusions. Positive ELISA results were similarly common for monovalent and trivalent vaccine recipients. Vaccine dose and baseline Ad5 immunity were major determinants of vaccine-induced seroconversion rates. Corresponding Western blots characteristically showed bands directed only at Gag proteins, which helped to distinguish HIV-uninfected vaccine recipients who experienced seroconversion from true HIV-infected patients. If available, an enzyme immunoassay exclusively targeting proteins not expressed by the vaccine should be the screening test of first choice for vaccine recipients.

The HIV pandemic continues to inflict devastating morbidity and mortality in many areas of the world [1, 2]. Safe and effective vaccines for both prevention and treatment are urgently needed [3, 4]. Developing an effective HIV vaccine still remains a formidable challenge [5–7]. Antibodies directed against Env proteins in glycoprotein (gp) vaccines do not seem to be protective [8]. Subsequent efforts that focus on inducing cell-mediated immunity against HIV with use of adenovirus type 5 (Ad5) vectors containing HIV-1 clade B transgenes have also culminated in disappointing results in a proof-of-concept study [5, 9–13]. Nonetheless, data from the Merck phase I program provide valuable information pertinent to the safety and tolerability of other potential HIV-1 vaccines that are being studied.

HIV-1 vaccines aimed at inducing cell-mediated immunity may also elicit antibodies to HIV proteins in some recipients. The resulting vaccine-induced HIV seroconversion detected by standard screening ELISA could pose a barrier to enrolling persons in vaccine trials and, eventually, to implementing successful vaccine programs [14–20]. Seroconversion rates will depend on the particular vaccine candidate and on the specific serological test. On Western blots, gag vaccines without env inserts would be expected to cause anti-
bodies reactive with Gag proteins (e.g., the Gag precursor protein p55 and the viral capsid protein p24) but not with proteins encoded by env (i.e., gp120/gp160 and gp41) (figure 1). Tests that might reliably distinguish true infection from vaccine-induced positive ELISA results potentially include Western blots, EIA exclusively measuring antibodies directed against epitopes not expressed by the vaccine, and nucleic acid amplification tests. Failure to use an appropriate testing algorithm for vaccine recipients and misinterpretation of ELISA results may lead to unnecessary distress and/or discrimination.

The objectives of the current analysis were to assess the frequency and characteristics of seropositivity induced by Merck Ad5-vectored HIV-1 vaccines and to differentiate seropositivity attributable to the vaccine from that attributable to natural infection. The high rate and long persistence of vaccine-induced seropositivity in our studies forewarn that postvaccination seroconversion may become a global public health issue hindering implementation of any HIV vaccine program. This hurdle needs to be anticipated and addressed. Our data further indicate that an insert-specific EIA can be useful as an HIV screening test if the EIA does not detect vaccine-induced antibodies.

**PARTICIPANTS, MATERIALS, AND METHODS**

To assess the rate of vaccine-induced seroconversion, we performed a retrospective analysis of the 3 following phase I trials of Ad5-vectored HIV-1 near-consensus clade B vaccines given to healthy, HIV-seronegative adult volunteers deemed to be at low risk of acquiring HIV infection during the studies: Merck Protocol 007 (monovalent Ad5 gag vaccine), Merck Protocol 012 (monovalent MRKAd5 gag vaccine), and Merck Protocol 016 (trivalent MRKAd5 gag/pol/nef vaccine) [14]. The studies were conducted in the United States. All uninfected participants who had received at least 1 dose of vaccine and had a sample tested by ELISA by week 78 were included in this post-hoc analysis. Serologic results were summarized by baseline Ad5 antibody titer (≤200 or >200), as well as by vaccine valency (monovalent gag or trivalent gag/pol/nef) and dose. Participants who developed HIV infection, defined by detectable plasma viral RNA by PCR assay, were excluded from the analysis.

**Vector and vaccine composition.** Two similar replication-defective Ad5 vectors were studied. The first vector studied was designated Ad5 HIV-1 gag. In preclinical studies, this vaccine appeared to be immunogenic and to have a safety profile that was acceptable for use in phase I trials, leading to Protocol 007, to study this vector in humans. After the initiation of Protocol 007, studies designed to support large-scale manufacturing of the Ad5 HIV-1 gag vaccine revealed a degree of genetic instability that would make the vector unsuitable for large-scale manufacturing. The same gag transgene was incorporated into a related Ad5 vector with a modified backbone, designated MRKAd5 HIV-1 gag [13, 22]. This modified vector was subsequently chosen for further development, because no significant genetic instability could be demonstrated. An admixture of MRKAd5 vectors containing gag, pol, and nef transgenes was used in Protocol 016 [13]. The gag inserts included in the monovalent and trivalent vaccines were identical.

**Study designs.** All 3 studies were phase I, placebo-controlled, multicenter, double-blind, randomized, dose-escalating trials. Eligible participants were healthy, HIV-seronegative adults aged 18–50 years who were considered to be at low risk of exposure to HIV infection. Participants had to be in good health, as determined by medical history and physical examination, and results of laboratory tests of blood chemistry, hematology, liver function, renal function, and clotting function.

![Illustrative Western blot patterns.](https://academic.oup.com/cid/article-abstract/47/12/1593/333269)
had to be within normal limits. Pregnant or breast-feeding women were excluded, as were persons with serological evidence of hepatitis B virus or hepatitis C virus infection. Women with childbearing potential were required to have a negative urine pregnancy test result immediately before each injection. Each study was approved by the institutional review boards of all participating centers. Written informed consent was obtained from all participants. The dose levels tested were 1 × 10^10, 1 × 10^11, and 1 × 10^12 viral particles (vp)/dose for the monovalent MRKAd5 gag vaccine; 1 × 10^9, 1 × 10^10, and 1 × 10^11 vp/dose for the monovalent MRKAd5 gag vaccine; and 3 × 10^6, 3 × 10^7, 3 × 10^8, 3 × 10^9, and 1 × 10^10 vp/dose for the trivalent MRKAd5 gag/pol/nef vaccine [13]. The number of gag-containing Ad5 particles was equal in the corresponding dose groups of the monovalent and trivalent vaccine, except for in the 1 × 10^9 vp/dose group, for which the trivalent vaccine contained one-third the amount of gag-containing Ad5 particles as the monovalent vaccine at this dose (and 3.33 times the amount of gag-containing Ad5 particles as the 1 × 10^10 and 3 × 10^9 vp/dose of the monovalent and trivalent vaccines, respectively).

Enrolled participants were randomized on the first study day to receive 1.0-ml injections of either placebo or vaccine in the deltoid muscle. Participants received a total of 3 doses, given at day 1, week 4, and week 26. The studies were designed to have a total duration of 5 years, during which participants were to have samples tested by HIV ELISA at weeks 78, 104, 156, and 208. However, HIV test results after week 78 were not available for many participants in Protocols 007 and 012 because of rollover into another phase I HIV vaccine trial after week 78. Each positive ELISA result automatically triggered a series of confirmatory tests, including Western blot, Env-EIA, and quantitative PCR assays.

**Laboratory assessments.** Unfractionated IFN-γ enzyme-linked immunosorbent spot assays were used to measure T cell responses as a marker of vaccine immunogenicity [13, 23, 24]. Serum samples were tested at baseline for their ability to inhibit Ad5 infection in a quantitative neutralization assay using a recombinant Ad5 strain [25].

At screening and during the study, participants’ samples were tested for HIV-1 with use of a US Food and Drug Administration–approved ELISA containing both HIV core and envelope antigens and/or an Env-specific EIA. The ELISA kits were the HIVAB HIV-1/HIV-2 (rDNA) EIA (Abbott) and the Genetic Systems HIV-1/HIV-2 PLUS O EIA (Bio-Rad). The Env-EIA rapid kits used in our studies were the Genetic Systems HIV-1/HIV-2 Peptide EIA (Bio-Rad), Uni-Gold Recombigen HIV (Trinity Biotech), and Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad). Seropositive specimens were reflexively tested by Western blotting and quantitative PCR (UltraSensitive Amplicor HIV-1 Monitor Test, version 1.5, with a lower limit of detection of 50 viral RNA copies/mL; Roche Diagnostics). Western blot results were interpreted according to Centers for Disease Control and Prevention criteria (figure 1) [21, 26].

**RESULTS**

Postvaccination ELISA results were available by week 78 for 406 (89%) of 455 participants who received at least 1 dose of vaccine (table 1). Of these 406 vaccine recipients, 392 (97%) received all 3 doses. After excluding 3 participants who became HIV infected, as determined by a positive PCR result, 165 participants (41%; 87 [53%] were men; median age, 35 years; range, 18–50 years) were ELISA positive but PCR negative up to week 78. Of the 165 vaccine recipients who had positive ELISA results, 163 (99%) completed the full 3-dose series. The frequency of seroconversion was unrelated to vaccine valency, inversely related to baseline Ad5 titers, and directly related to vaccine dose, progressively increasing from 1 × 10^10 to 1 × 10^11 gag-containing Ad5 particles per dose (figure 2). Among 95 uninfected participants who received a dose of 10 × 10^10 gag-containing Ad5 particles per dose, 36 (63%) of 57 monovalent vaccine recipients and 24 (63%) of 38 trivalent vaccine recipients had a positive ELISA result, including 49 (89%) of 55 with low baseline Ad5 antibody titers and 11 (28%) of 39 with high baseline Ad5 titers. Overall, 100 (88%) of the 113 evaluable participants with low baseline Ad5 titers (=200) who received at least 1 dose of ≧1 × 10^10 gag-containing Ad5 particles per dose experienced seroconversion but were not HIV infected, as defined by a quantitative PCR assay. Of 163 ELISA-positive vaccine recipients with available Western blot interpretations, 150 (92%) had indeterminate results according to Centers for Disease Control and Prevention criteria. Among indeterminate Western blots available for inspection, bands present in ≧95% of trivalent vaccine recipients were p24, p40, and p55. Equivocally positive Western blot results were seen for 13 uninfected participants (8%; 12 monovalent vaccine recipients and 1 trivalent vaccine recipient, all of whom had received doses containing ≧1 × 10^10 gag-containing Ad5 particles per dose), usually because of an additional weak band at gp41. Among the participants who had positive ELISA results at week 78, ELISA positivity persisted through week 156 for the majority of evaluable participants who received vaccine doses containing ≧1 × 10^10 gag-containing Ad5 particles per dose (figure 3).

Env-EIA results were falsely positive for 2 (0.4%) of 498 tested participants (432 vaccine recipients and 66 placebo recipients) by week 78; both of these participants were vaccine recipients who had positive ELISA results and indeterminate Western blot results (without Env bands) for the same samples. Neither participant had detectable viremia by an ultrasensitive PCR assay. Each participant had multiple negative Env-EIA results before the single positive result; for 1 participant, the
result of an additional Env-EIA, performed after the test that yielded a positive result, was negative.

**DISCUSSION**

Vaccination against HIV-1 may be associated with seroconversion detectable by routine screening tests for HIV infection [14]. All ELISA assays currently approved by the US Food and Drug Administration for the diagnosis of HIV-1 infection use recombinant HIV-1 env- and gag-encoded proteins, such as gp160 and p24, respectively. We observed frequent and durable ELISA positivity after receipt of the Merck Ad5-vectored clade B HIV-1 vaccine candidates. In our summaries, combined across 3 protocols, vaccine-induced seroconversion rates for both the monovalent and trivalent vaccine were driven by antibodies directed against HIV-1 Gag proteins. Vaccine dose and baseline Ad5 titer had a significant impact on the frequency of vaccine-induced ELISA positivity. Seroconversion rates progressively increased with increasing vaccine dose. Relative to the higher-dose groups, positive ELISA results developed more commonly after vaccination for participants with baseline Ad5 titers \textless; 200 than for participants with baseline Ad5 titers \textgreater; 200. Among recipients of the highest vaccine doses, seropositivity rates at weeks 104 and 156 remained similar to the rate at week 78, regardless of baseline Ad5 titer, indicating that positive ELISA results at week 78 remained persistently positive for years after receipt of the final vaccine dose.

Western blot banding patterns for samples from uninfected participants who experienced seroconversion confirmed that antibodies to Gag proteins, particularly the p55 Gag precursor protein, the p24 capsid protein, and the p40 partial cleavage product of the p55 Gag precursor, were the primary cause of ELISA and Western blot reactivity [27]. The p40 band may be misinterpreted as representing the gp41 Env band, because variable glycosylation patterns on gp41 usually cause this band to appear as a broad, indistinct “smear.” Because env was not among the transgenes included in the Merck vaccines, Env-EIA results were almost uniformly negative. Two participants had false-positive Env-EIA results after vaccination, despite the absence of Env bands on the corresponding Western blots.
The algorithm used to determine the presence or absence of true HIV infection in vaccine recipients should begin with a specific EIA directed at HIV-1 proteins not included in the vaccine, if possible. Use of a vaccine-tailored, insert-specific EIA would avoid cross-reactivity between vaccine and natural infection and serve as an unconfounded screening test for HIV infection for HIV vaccine recipients. This testing paradigm may require development of novel EIA tests for global use to differentiate vaccine-induced seropositivity from true infection, once we have an effective HIV vaccine.

Unlike Env-based tests, ELISA using viral lysates or recombinant core proteins would be anticipated to cross-react with the Merck vaccines used in these studies. Our findings indicate that HIV-1 infection can be confidently excluded in persons who have already tested positive by standard ELISA after vaccination on the basis of the characteristic vaccine-induced banding pattern on follow-up Western blots (showing a p24 band, generally with p40 or p55 bands). An Env-specific EIA and a quantitative PCR assay for viral RNA would also have been expected to accurately distinguish between true infection and vaccine-induced seropositivity in those vaccine recipients in our studies who developed positive ELISA results.

Failure to use an appropriate testing algorithm for vaccine recipients, compounded by misinterpretation of the results of routine ELISA, could inflict avoidable social harm and unnecessary emotional hardship [15–20]. Possible problems obtaining medical or dental insurance, gaining employment, traveling abroad, or entering military service may dissuade participants from entering vaccine trials. An earlier review of serological testing after receipt of poxvirus-vectorized HIV vaccines and/or recombinant Env-protein subunit vaccines highlighted several implications of a relatively low misclassification rate of 20% among uninfected vaccine recipients [14]. The frequency of seroconversion after vaccination depends on the particular vaccine being used [8, 13, 23–25]. The rates encountered in our vaccine trials were substantially higher than those reported elsewhere [14].
Distinguishing vaccine-induced seroconversion from true HIV infection is critically important and ultimately feasible. When possible, HIV testing algorithms consisting of ELAs based exclusively on antigens not expressed in the vaccine under study should be the screening assay of choice for vaccine recipients. Although these tests have high specificity (i.e., low rates of false-positive results), the predictive value of a positive result can be compromised when the test is applied to a low-risk population. If a standard ELISA has already been performed and has yielded a positive result, Western blot pattern analysis (supplemented by additional serological testing and judicious use of ultrasensitive PCR assays in individualized circumstances) can reliably distinguish vaccine-induced seropositivity from actual HIV-1 infection. Selection of appropriate serological tests will need to be tailored to the actual composition of the specific vaccine administered [8, 13, 14, 28–30]. Participants in HIV vaccine trials and their caregivers should be aware of the possibility of at least transient vaccine-induced seropositivity and the appropriate HIV screening tests to use, to reduce the chance of misleading (and distressing) positive results [15–20]. Novel serological tests that rely on antibodies that are not likely to be elicited by HIV-1 vaccines are in development [31], and optimization of nucleic acid assays for diagnostic use (based on transcribed DNA rather than on viral RNA) is also underway [32].

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