Long-Term Persistence of Vaccine-Induced HIV Seropositivity among Healthy Volunteers

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Abstract

Long-term persistence of HIV vaccine-induced seropositivity in uninfected HIV vaccine recipients remains unknown. The duration of HIV humoral-induced responses was assessed in 72 volunteers who had received rgp160 and/or HIV recombinant canarypox virus constructs able to induce immune responses detectable using standard serological tests. Among the 43 rgp160 recipients, 94% and 83% remained HIV seropositive after 5 and 8 years of follow-up, respectively, while all the 29 volunteers who had received canarypox constructs alone were seronegative after 5 years. Because rgp160 induces long-term persistence (>8 years) of vaccine-induced HIV seropositivity, volunteers should be offered long-term follow-up to monitor their serological evolution.

Since 1987, more than 35 candidate vaccines have been assessed in over 65 phase I/II vaccine clinical trials involving >10,000 healthy volunteers.1 Some of these vaccines express all or part of the Gag, Pol, Env, and/or Nef proteins that can induce humoral immune responses, detectable on standard serological tests that can be interpreted as positive and thereby mistakenly indicate HIV infection.2–4 Nonetheless, the duration of vaccine-induced HIV seropositivity remains undetermined. The aim of this study was to evaluate the persistence of positive HIV serology induced by different candidate HIV-1 vaccines.

From 1992 to 1997, the French National Agency for AIDS Research and viral hepatitis (ANRS) conducted six phase I HIV-1 vaccine trials in France focused on the quality, specificity, and duration of immune responses following immunizations with vaccine constructs including recombinant envelope protein subunit (rgp160) and/or canarypox virus (vCP) construct(s) coding for HIV-1 envelope and core proteins. Two trials (ANRSVAC01 and ANRSVAC02) administered rgp160 with an adjuvant (incomplete Freund’s adjuvant or aluminum hydroxide), three trials (ANRSVAC01, ANRSVAC05, and ANRSVAC06) used ALVAC-HIV (vCP125), a construct expressing gp160 (MN strain), one trial (ANRSVAC03) used ALVAC-HIV (vCP205), which expresses gp120 MN tm-LAI/gag/protease-LAI, and one trial (ANRSVAC07) administered ALVAC-HIV (vCP300), expressing gp120 MN tm-LAI/gag/protease and pol and nef CTL domains. All vaccines were injected intramuscularly.

Healthy volunteers 21–55 years old were prescreened according to the procedure established by ANRS to select HIV-unexposed subjects, as previously described.5 They were counseled regarding the need to avoid HIV exposure and received no financial incentive. All the volunteers were informed that there was no evidence that the vaccine product had any protective effect against HIV infection, and that they might even become more susceptible to infection. They were also informed of the potential psychological and social consequences of HIV seropositivity notably due to the devel-

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 closure of anti-gp160 antibodies. At the end of the vaccine trial, the volunteers were systematically asked to continue their participation in a long-term follow-up study consisting of a physical examination and blood sample for HIV testing. For volunteers with negative HIV serology at the end of the trial, follow-up was scheduled every 2 years for 6 years. For those with vaccine-induced HIV seropositivity, follow-up was scheduled yearly until HIV serology became negative, and every 2 years thereafter for 6 years.

Among the 75 volunteers who have received at least one injection of rgp160 and/or vCP constructs, 72 (96%) gave their written consent to participate in the long-term follow-up study: 29 received vCP205 or vCP300, 17 received rgp160 alone, and 26 received vCP125 and rgp160 sequentially.

At each follow-up visit, anti-HIV antibodies were detected using two different licensed enzyme immunoassays (EIAs). Since volunteer follow-up spanned several years, different screening kits were used: Abbott (Abbott HIV1/HIV2 EIA third-generation plus) and Organon (Vironostika HIV uniform II) between 1992 and 1997, Ortho (HIV1/HIV2 enhanced ELISA) and Organon (Vironostika HIV uniform II) between 1998 and 2000, and Genscreen HIV1/2 (Bio-Rad) and Enzygnost HIV Integral (Dade Behring) between 2000 and 2002. As of 2003, Genscreen HIV1/2 (Bio-Rad) and Murex HIV Ag/Ab Combo (Abbott Murex) were used. EIAs were performed according to the manufacturer’s specifications. All sera were also tested by Western blot (WB) regardless of the EIA result. A positive WB was defined as the presence of any two bands identifying gp41, gp120/160, and/or p24; an indeterminate WB was defined as the presence of band(s) that did not meet the criteria defining positivity, and a WB was considered negative when no band was present (criteria used by the Association of Public Health Laboratories/CDC). A volunteer was considered to be vaccine-induced seropositive when at least one of the two screening EIA was positive, regardless of the WB result.

The follow-up analysis was designed to determine the impact of the vaccine constructs and vaccine schedules on the time until EIA became negative. A time-to-event analysis was conducted with nonparametric (Kaplan–Meier) and parametric approaches. Time zero was defined as the last vaccine injection, regardless of the number of injections received by the volunteer. The time to EIA negativity was first analyzed by the Kaplan–Meier method, with three strata according to the type of vaccination administered: (1) rgp160 alone (n = 17), (2) rgp160 plus vCP125 (n = 26), or (3) vCP205 or vCP300 (n = 29). The overall significance was assessed with a chi-square test. These analyses were conducted with S-plus 4.5 software. The time to EIA negativity in strata 1 and 2 was then subjected to a parametric analysis. An exponential survival distribution (Weibull) was assumed, with shape parameter $r$ and median survival $m_i$, depending on the pattern of vaccine injections for individual $i$. Median survival was assumed to be given by $m_i = b_1, N_{gp160} + b_2, N_{vCP125} + b_3, ind$, where $N_{gp160}$ and $N_{vCP125}$ are the numbers of injections of each vaccine type, $ind$ is a dummy variable indicating that vCP125 was injected after rgp160, and $\beta$ represent unknown regression coefficients corresponding to the additional time to EIA negativity per injection of each antigen. Although no subject received vCP125 alone, $\beta_2$ could be estimated because it accounts for the difference between the two strata. According to the value of $r$, the risk of becoming seronegative could be constant ($r = 1$), higher ($r > 1$), or lower ($r < 1$) over time. Individuals who became seronegative <1 month after the last vaccine injection were treated as left censored, while those who were not yet seronegative at the last follow-up visit were treated as right censored. The posterior distribution of the parameters ($r$, $\beta$) and medians $m_i$ were determined by Monte Carlo–Markov Chain simulation using WinBugs 1.4.8

Among the 72 volunteers included in this study, 25 (35%) were female. At enrollment in the trials, their median age was 41.5 years (range, 27–56) and their median weight was

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**FIG. 1.** HIV Western blot profile during long-term follow-up according to vaccine construct.
72.5 kg (range, 47–94). The median duration of long-term follow-up was 8.5 years (range, 1–12). One volunteer who developed an ovarian tumor discontinued follow-up and 12 (17%) volunteers were lost to follow-up. None of the volunteers developed HIV infection.

At the end of their participation in the vaccine trial, 62 (86%) subjects had developed vaccine-induced HIV seropositivity: all the 43 subjects who had received rgp160 alone or combined with vCP125 and 19 (65.5%) of the 29 subjects given vCP300 or vCP205 alone. After 5 years of follow-up, 34 (94%) of the 36 volunteers who had received rgp160 were still HIV seropositive, as opposed to none of those given vCP300 or vCP205 alone. Finally, after 8 years of follow-up, only 6 (17%) of the remaining 35 rgp160 recipients had become seronegative. The percentages of volunteers with positive, intermediate, or negative WB results at each time are presented in Fig. 1.

No significant relationship was found between sex and/or weight and duration of vaccine-induced HIV seropositivity. The influence of age (stratified as <42 years or ≥42 years) on the duration of positive HIV serology could not be assessed because of too few participants in each stratum. The Kaplan–Meier analysis showed that the time to seronegativity was longer (p < 0.0001) for volunteers who had received rgp160 alone or in combination with vCP125 than those given vCP205 or vCP300 (Fig. 2). However, the median time to EIA seronegativity for the first two strata could not be calculated by this approach because <50% of volunteers had become seronegative. To overcome this obstacle, a parametric method was applied to analyze the data from the first two strata. This analysis showed that the risk of becoming seronegative remained constant over time (r was not significantly different from 1) and that the time to EIA seronegativity was not affected by the order of rgp160 and vCP125 injections (β₁ was not significantly different from 0). β₁ and β₂ values showed that the median durations of the immune responses per injection of rgp160 or vCP125 alone were 82 and 28 months, respectively.

The first HIV trials evaluated envelope-based vaccine candidates, such as recombinant gp160 protein. These vaccine candidates were highly immunogenic but unfortunately were unable to induce neutralizing antibodies. In light of the difficulties in eliciting neutralizing antibodies, and because numerous studies have provided evidence of the role of T cell-mediated immunity in controlling HIV infection, especially cytotoxic CD8⁺ T cells, there has been considerable interest in vaccines inducing T cell responses. Some of these T cell vaccine candidates, among which are vCP constructs, are able to induce humoral immunogenicity and vaccine-induced seropositivity.

The rate of HIV vaccine recipients developing humoral immune responses detectable by standard serological tests after vaccination varied greatly with the candidate HIV vaccine tested. In this study, all the volunteers who received rgp160 became vaccine-induced HIV seropositive, as opposed to only 65% of those who received only vCP300 or vCP205. In another study, the analysis of sera collected 2 weeks after the last vaccine injection from volunteers who participated in AIDS Vaccine Evaluation Group trials (AVEG) showed that vaccine-induced HIV antibodies was evidenced in 90–100% of the sera from rgp160 recipients (injected with vCP constructs), but only 16% and 9% of those given vCP125 or vCP300 alone, respectively. These discrepancies might be explained by the different vaccination schedules used and the numbers of injections given.

The long-term persistence of HIV vaccine-induced humoral response is unknown. We showed a persistent vac-
cine-induced HIV seropositivity more than 8 years in volunteers who had received gp160 and between 1 and 5 years in volunteers receiving vCP constructs. No correlation between sex or weight and the persistence of humoral responses was identified, but the number of subjects was low. In another study, four of the six volunteers given vCP250 or vCP300 boosted with rgp120 in AVEG trials had measurable titers of MN-neutralizing antibodies 5 years after receiving vaccines.9

As the rate and long-term persistence of HIV vaccine-induced humoral response depend on vaccine construct, it will be of interest to carry out identical analysis on recent and future vaccine constructs such as DNA. Actually, a DNA prime-protein boost approach appears to be an effective immunization method to elicit both humoral and cell-mediated immune responses in humans, and to generate broad immune responses against HIV-1 viruses with diverse genetic backgrounds.10

Persistent long-term humoral responses in HIV vaccine recipients may have several implications for clinicians and volunteers. Vaccine recipients may be mistakenly identified as being HIV infected based on their serological EIA or rapid test results up to 8 years after the study’s end.11 In such cases, WB and/or polymerase chain reaction analyses can eliminate diagnostic errors. However, EIA positivity may carry social consequences.12–14 Moreover, these individuals can no longer donate their blood.13 Social problems were reported by volunteers of the ANRS network, but this information was not collected until now. The evaluation of the long-term safety of vaccine candidates and the psychosocial behavioral consequences of participation in HIV vaccine trials are ongoing in France.15

In conclusion, volunteers must be informed prior to trial enrollment of the risk of persistent vaccine-induced HIV seropositivity and the potential psychological and social consequences. They should be offered long-term follow-up to monitor their serological evolution and to evaluate the consequences of their participation in such trials.

Acknowledgments

The authors wish to thank the volunteers who generously gave their time and themselves, Janet Jacobson for editing the English text, and Martine Renaud for her help in collecting data. All vaccine trial participants provided written informed consent for human immunodeficiency virus testing and trial participation, and the study protocol was approved by the Cochin Hospital Ethics Committee, Paris, France. Volunteer follow-up is sponsored by Sanofi Pasteur and the French National Agency for AIDS Research and Viral Hepatitis (Agence Nationale de Recherche sur le SIDA et les Hépatites Virales ANRS). The first two authors contributed equally to this study.

Disclosure Statement

No competing financial interests exist.

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