Published in final edited form as:

J Acquir Immune Defic Syndr. 2007 April 15; 44(5): 601–605.

Safety and Immunogenicity of a Gag-Pol Candidate HIV-1 DNA Vaccine Administered by a Needle-Free Device in HIV-1–Seronegative Subjects

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Abstract

Objective—To evaluate the safety and immunogenicity of a candidate HIV DNA vaccine administered using a needle-free device.

Design—In this phase 1, dose escalation, double-blind, placebo-controlled clinical trial, 21 healthy adults were randomized to receive placebo or 0.5, 1.5, or 4 mg of a single plasmid expressing a Gag/Pol fusion protein. Each participant received repeat immunizations at days 28 and 56 after the first inoculation. Safety and immunogenicity data were collected.

Results—The vaccine was well tolerated, with most adverse events being mild injection site reactions, including pain, tenderness, and erythema. No dose-limiting toxicities occurred. HIV-specific antibody response was not detected in any vaccinee by enzyme-linked immunosorbent assay. HIV-specific T-cell responses to Gag or Pol as measured by enzyme-linked immunospot assay and intracellular cytokine staining were of low frequency and magnitude.

Conclusions—This candidate HIV DNA vaccine was safe and well tolerated. No HIV-specific antibody responses were detected, and only low-magnitude HIV-specific T-cell responses were detected in 8 (53%) of 15 vaccinees. This initial product led to the development of a 4-plasmid multiclade HIV DNA Vaccine Research Center vaccine candidate in which envelope genes expressing Env from clades A, B, and C and a Nef gene from clade B have been added.

Keywords

CD4⁺ T-cell immune response; gene delivery; immunization; needle-free device; plasmid vaccine; safety

More than 40 million people worldwide are infected with HIV-1, and as many as 14,000 new infections occur each day.^{1,2} Development of a safe and effective HIV-1 vaccine is essential to controlling the HIV/AIDS pandemic.

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DNA vaccination induces CD4⁺ and CD8⁺ T-lymphocyte responses. Another advantage is the lack of antivector immunity to the plasmid.^{3,4} HIV DNA vaccination has elicited detectable humoral and T-cell-mediated immune responses in numerous animal studies.^{5–8} DNA vaccination was first shown to be immunogenic in antigen-naïve humans in vaccine programs for malaria and hepatitis B.^{9,10} An early trial of HIV-1 DNA vaccination for Env and Rev in HIV-seronegative adults was safe and produced modest HIV-specific T-cell responses.¹¹ In recent studies evaluating newer generation Vaccine Research Center (VRC) DNA vaccines, however, a VRC HIV DNA 4-plasmid vaccine expressing envelope from clades A, B, and C and Gag, Pol, and Nef from clade B demonstrated improved cellular and humoral responses to the envelope antigens but limited immunogenicity to internal proteins,¹² and a VRC Ebola DNA vaccine was recently shown to be safe and immunogenic in all vaccinees.¹³

We present the safety and immunogenicity of a phase 1 clinical trial of the first VRC candidate HIV-1 DNA vaccine construct encoding Gag and Pol administered intramuscularly by means of a Biojector 2000 Needle-Free Injection Management System (Tualatin, Oregon) to healthy adults, the prelude to the VRC 4-plasmid vaccine candidate.

MATERIALS AND METHODS

Vaccine Construct and Administration

The DNA vaccine pVRC4302, or pGag (del fs)PolΔRTΔInt/h, expresses HIV Gag and Pol proteins. The safety of this vector is enhanced by 2 major design features: (1) elimination of the viral long terminal repeat (LTR) to eliminate the possibility of packaging and spread of the introduced sequence and (2) inclusion of 3 independent point mutations in the protein coding regions that affect protease, reverse transcriptase, and integrase, thus eliminating the probability of single revertant producing a biologically active particle. The vaccine was in phosphate-buffered saline (PBS) solution and was administered as an intramuscular injection using the US Food and Drug Administration (FDA)–cleared Biojector 2000 Needle-Free Injection Management System. PBS was administered as a placebo.

Study Design

After receiving National Institute of Allergy and Infectious Diseases (NIAID) Institutional Review Board approval and informed consent, 21 healthy HIV-seronegative subjects with normal renal, hepatic, and hematologic laboratory parameters were enrolled at the Clinical Center of the National Institutes of Health (NIH) from May 2001 through May 2003. Pharmacy staff implemented the block allocation randomization sequence, and study investigators and subjects remained blinded to randomized allocation. Each subject received immunizations at days 0, 28, and 56. Three sequential dose groups were enrolled. Safety of the prior dose was evaluated before dose escalation. Each group included 5 vaccine recipients and 2 placebo recipients. Total enrollment comprised 21 subjects: 5 subjects each received 0.5 mg, 1.5 mg, or 4 mg of DNA vaccinations, and 6 subjects received placebo injections. After the safety of the 0.5-mg dose was established, the next 7 subjects were randomized to receive 1.5 mg of DNA vaccine (5 subjects) or PBS placebo (2 subjects). Finally, after the safety of this dose was established, the next 7 subjects were randomized to receive 4.0 mg of DNA vaccine (5 subjects) or PBS placebo (2 subjects).

Safety and immunologic assessments were performed on injection days; 2 weeks after each injection; and at weeks 24, 38, and 52 after the initial injection.

Immunologic Assays

T-Cell Analyses—Vaccine-induced cellular immune responses were measured by extensively validated methods, including intracellular cytokine staining (ICS) and enzyme

immunospot (ELISpot) assay on cryopreserved peripheral blood mononuclear cells (PBMCs) at baseline and weeks 10, 12, 24, and 52 as previously described.¹² The 4-parameter flow cytometric ICS analysis evaluated CD3, CD4, CD8, and interferon- γ (IFN γ) or interleukin-2 (IL-2) simultaneously, whereas the ELISpot assay used an IFN γ endpoint on unfractionated PBMCs as previously described.¹² The criteria for positivity for each of these assays has been previously described.¹²

Enzyme-Linked Immunosorbent Assays—In addition to an FDA-approved enzyme-linked immunosorbent assay (ELISA), research ELISAs were performed to evaluate the antibody response to individual viral antigens encoded within the vaccine. Endpoint titers of antibodies directed against HIV antigens were assessed as described previously.^{12,14}

Statistical Analyses—The primary endpoint of safety was addressed by examining adverse events and reactions among vaccinees compared with placebo recipients. Study sample size was chosen to detect large differences between the control ($n = 6$) and combined vaccine recipient ($n = 15$) groups. With a 1-sided test, level of significance of 0.05, and power of 80%, the difference between a 3% control group severe reaction rate and a 49% treatment group severe reaction rate is detectable.

T-cell responses to HIV epitopes were measured. A difference of approximately 41% HIVepitope-specific activity between placebo (1%) and vaccine (42%) groups can be detected with a 1-sided test at a level of significance of 0.05 and power of 0.80.

RESULTS

Study Population

The demographics of the study subject are shown by treatment group in Table 1. Fifty-two percent of study subjects were female. The mean age of subjects was 40 years.

Study Follow-Up

Vaccinations were completed in 21 (100%) of 21 subjects, and data from all subjects are included in the analyses. Subjects were monitored for clinical adverse events, reactogenicity, laboratory abnormalities, pregnancy, and HIV infection through 52 weeks of follow-up.

Vaccine Safety

Vaccinations were well tolerated with no dose-limiting toxicities. Vaccine recipients had mild local induration, pain, and erythema, with no suggestion of a dose effect. Injection site local reactogenicity was measured in all subjects. The mean maximum diameter of erythema in the vaccinees was 1.4 cm, with a range of 0 to 6 cm. The mean maximum diameter of erythema in the placebo recipients was 0.03 cm, with a range of 0 to 0.2 cm. The mean maximum diameter of induration in the vaccinees was 0.4 cm, with a range of 0 to 3 cm. There was no consistent trend in reactogenicity related to dose or number of vaccinations received. None of the placebo recipients recorded measurable induration. Intramuscular injection with the Biojector device results in minimal local reactogenicity, and this likely explains the mild local injection site findings. Table 2 shows adverse events by Medical Dictionary for Regulatory Activities (MedDRA) code for placebo and vaccine subjects. A breast ductal carcinoma in situ in a 0.5-mg recipient was diagnosed 209 days after the third vaccination. This case was independently reviewed by an oncologist. The attribution was unlikely to be related to the study agent and was possibly a preexisting condition.

Evaluation for antinuclear antibody (ANA) and anti-double-stranded DNA (dsDNA) occurred on the initial visit and at time points throughout the duration of the study. One individual in

the 0.5-mg dose group had a weakly positive ANA of 1.6 ELISA Unit (EU), initially seen after the first vaccination, and was intermittently weakly positive after the third vaccination. The ANA returned to negative and remained negative throughout the follow-up period of the study. Anti-dsDNA antibody did not develop in any study subject.

T-Cell Responses

Eight (53%) of 15 vaccinees developed a transient low-magnitude T-cell response to Pol or Gag peptide as detected by ICS or ELISpot assay. Six of the responders were positive for Gag or Pol peptide stimulation by ICS or ELISpot assay at only a single time point. One subject in the 0.5-mg dose group had an unexplained CD4 response that met criteria for positivity by ICS and ELISpot assay to Gag at baseline. There was no measurable effect on that subject's CD4 response after vaccination, and those values are not included in the analysis. That individual did not have a positive ELISA result, did not have HIV infection by polymerase chain reaction (PCR), and had no known history of HIV risk factors; the low-magnitude responses were considered to be falsely positive. Only 1 subject (4-mg dose group) developed a CD4 response by ICS to Gag and Pol and an ELISpot response to Gag. This 4-mg recipient is the only responder with concordant ICS and ELISpot responses to the same peptide. The frequency and magnitude of T-cell responses in vaccinees by dose group are shown in Figure 1.

Antibody Responses

No vaccine-induced antibody was detected by endpoint ELISA or commercial ELISA at week 10, 12, 24, or 52.

DISCUSSION

This single-plasmid HIV DNA vaccine construct was found to be safe and well tolerated in healthy HIV-seronegative adults. This VRC HIV DNA candidate vaccine was the first product tested in a phase 1 clinical trial by the VRC, NIAID, NIH.

In this DNA vaccine clinical trial initiated in 2001, subjects were routinely assessed for the development of ANA and anti-dsDNA, as was common in early DNA vaccine studies. Important findings in this trial were that anti-dsDNA antibodies were not present in any subject at any time point and only a single subject in the lowest dose vaccine group had a transient low level of ANA. These findings corroborate the findings of other studies demonstrating that DNA vaccination does not seem to be associated with the development of ANA or anti-DNA antibodies and should not be routinely monitored throughout a DNA vaccine clinical trial.^{10, 15,16} It is also noted that the FDA February 2005 "Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Diseases" (DRAFT), states that preclinical studies have helped to establish that systemic autoimmunity is unlikely to result from DNA vaccines.

The vaccine-induced T-cell responses were of low magnitude, and only 1 subject developed a response to an HIV antigen that could be measured by the ICS and ELISpot methods. At least 1 HIV-specific T-cell response occurred in 53% (8 of 15) of vaccinees.

Although the safety and tolerability of this DNA vaccine were acceptable and this study further supports the principle that DNA vaccination is safe, the modest immunogenicity of this vaccine motivated the development of a more complex 4-plasmid HIV-1 vaccine construct in which the addition of Env antigens from clades A, B, and C produced a much higher level of immunogenicity in a recently completed phase 1 clinical trial.¹²

ACKNOWLEDGMENTS

The authors thank the study volunteers who gave their time and understand the importance of finding a safe and effective HIV vaccine. They also thank NIH Clinical Center staff, NIAID staff, NIAID Outpatient Clinic 8 Staff,

Public Recruitment and Public Liason Office and the Office of Communications and Public Liaison staff, Biojector (Richard Stout), Vical (Jennifer Meek), and VRC supporting staff (Richard Jones and Monique Young) who made this work possible.

The VRC 001 study team consists of Drs. H. Clifford Lane, Henry Masur, Richard Davey, and Michael Polis.

Supported by intramural funding from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

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FIGURE 1.

T-cell responses of all vaccinees. Gag and Pol CD4- and CD8-specific T cells were assessed by ICS and ELISpot assay. Open circles represent the 0.5-mg dose recipients, triangles represent the 1.5-mg dose recipients, and closed circles represent the 4-mg dose recipients at their peak-magnitude response to the given assay throughout the study. CD4 ICS responses >0.0241% were considered positive. CD8 ICS responses >0.0445% were considered positive. ELISpot assay responses >59 spot forming cells (SFC) per million PBMCs were considered positive. The peak response was seen at a mean of week 17.

TABLE 1

Demographics of All Study Subjects

Demographic	All Vaccinees (n = 15)	Placebo (n = 6)	All Subjects (n = 21)
Gender			
Male	9 (60%)	1 (17%)	10 (48%)
Female	6 (40%)	5 (83%)	11 (52%)
Mean age in years [range]	40.4 [23–58]	37.7 [25–48]	39.6 [23–58]
Race/ethnicity			
White, not Hispanic	14 (93%)	4 (66.7%)	18 (85.7%)
Black, not Hispanic	0	0	0
Asian	0	1 (16.7%)	1 (4.8%)
Hispanic	1 (7%)	1 (16.7%)	2 (9.5%)

TABLE 2
Adverse Events by Body System in Vaccine and Placebo Recipients

Body System MedDRA Term	Vaccine (n = 15)	Placebo (n = 6)
Number with 1 or more AE	14 (93.3%)	6 (100.0%)
Blood and lymphatic system disorders	3 (20.0%)	1 (16.7%)
Anemia NOS	1 (6.7%)	0 (0.0%)
Leukocytosis	2 (13.3%)	1 (16.7%)
Gastrointestinal disorders	3 (20.0%)	0 (0.0%)
Diarrhea NOS	1 (6.7%)	0 (0.0%)
Gastroesophageal reflux disease	1 (6.7%)	0 (0.0%)
Hemorrhoids	1 (6.7%)	0 (0.0%)
General disorders and administration site condition	1 (6.7%)	1 (16.7%)
Fatigue	1 (6.7%)	0 (0.0%)
Pyrexia	0 (0.0%)	1 (16.7%)
Infections and infestations	6 (40.0%)	0 (0.0%)
Gastroenteritis NOS	1 (6.7%)	0 (0.0%)
Upper respiratory tract infection NOS	5 (33.3%)	0 (0.0%)
Urinary tract infection NOS	1 (6.7%)	0 (0.0%)
Vaginitis	1 (6.7%)	0 (0.0%)
Injury, poisoning and procedural complications	1 (6.7%)	0 (0.0%)
Laceration	1 (6.7%)	0 (0.0%)
Investigations	11 (73.3%)	3 (50.0%)
Activated partial thromboplastin time prolonged	1 (6.7%)	0 (0.0%)
Alanine aminotransferase increased	3 (20.0%)	1 (16.7%)
Aspartate aminotransferase increased	4 (26.7%)	1 (16.7%)
Blood bilirubin increased	5 (33.3%)	1 (16.7%)
Blood creatine phosphokinase increased	3 (20.0%)	1 (16.7%)
Prothrombin time prolonged	1 (6.7%)	0 (0.0%)
Weight decreased	2 (13.3%)	1 (16.7%)
Weight increased	1 (6.7%)	0 (0.0%)
Metabolism and nutrition disorders	7 (46.7%)	4 (66.7%)
Hypercalcemia	0 (0.0%)	1 (16.7%)
Hypercholesterolemia	0 (0.0%)	1 (16.7%)
Hyperglycemia NOS	5 (33.3%)	0 (0.0%)
Hypoalbuminemia	3 (20.0%)	1 (16.7%)
Hypoglycemia NOS	2 (13.3%)	0 (0.0%)
Hyponatremia	0 (0.0%)	1 (16.7%)
Musculoskeletal and connective tissue disorders	3 (20.0%)	1 (16.7%)
Arthralgia	0 (0.0%)	1 (16.7%)
Muscle cramps	1 (6.7%)	0 (0.0%)
Myalgia	1 (6.7%)	1 (16.7%)
Neck pain	1 (6.7%)	0 (0.0%)
Tendonitis	1 (6.7%)	0 (0.0%)
Nervous system disorders	1 (6.7%)	0 (0.0%)
Headache NOS	1 (6.7%)	0 (0.0%)
Psychiatric disorders	1 (6.7%)	1 (16.7%)
Anxiety	1 (6.7%)	0 (0.0%)
Depression	0 (0.0%)	1 (16.7%)
Renal and urinary disorders	6 (40.0%)	2 (33.3%)
Hematuria	4 (26.7%)	1 (16.7%)
Proteinuria	2 (13.3%)	1 (16.7%)
Pyuria	2 (13.3%)	1 (16.7%)
Respiratory, thoracic and mediastinal disorders	2 (13.3%)	2 (33.3%)
Cough	0 (0.0%)	1 (16.7%)
Dyspnea NOS	2 (13.3%)	0 (0.0%)
Laryngitis NOS	0 (0.0%)	1 (16.7%)
Pharyngitis	0 (0.0%)	1 (16.7%)
Skin and subcutaneous tissue disorders	2 (13.3%)	0 (0.0%)
Dermatitis contact	1 (6.7%)	0 (0.0%)
Folliculitis	1 (6.7%)	0 (0.0%)
Rash NOS	1 (6.7%)	0 (0.0%)
Vascular disorders	4 (26.7%)	0 (0.0%)
Hypertension NOS	3 (20.0%)	0 (0.0%)
Hypotension NOS	1 (6.7%)	0 (0.0%)

Percentages represent the number of participants with the given event divided by the total number of participants.

For participants reporting multiple experiences within a row, the experience type is counted once as the maximum severity.

AE indicates adverse event; NOS, not otherwise specified.