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Phase I Clinical Trial with HIV-1 gp160 Plasmid Vaccine in HIV-1-Infected Asymptomatic Subjects

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Abstract The aim of the study was to investigate the safety of an HIV-1 gp160 plasmid vaccine. Four asymptomatic HIV-1-infected subjects with CD4+ lymphocyte counts >500/μl were injected with four times 400 μg of HIV-1 modified gp160 *env* and *rev* coding DNA vaccine at 0, 4, 10 and 28 weeks. Safety parameters, including autoimmune antibodies as well as CD4+/CD8+ cell counts and HIV-1 plasma concentrations, were monitored for 52 weeks after the first vaccine application. Follow-up data for more than 3 years are now available. The DNA vaccine proved to be safe and, specifically, did not induce anti-DNA autoimmune antibodies. Vaccination had no long-term effects on the CD4+/CD8+ lymphocyte counts, plasma HIV-1 RNA concentrations or disease progression. The present data supplement published data from Philadelphia, USA, where a dose-escalating study (30–300 μg) with the same HIV-1 DNA vaccine was performed.

Introduction

Genetic vaccination represents an alternative approach towards vaccine development for a variety of diseases. HIV-1 DNA constructs in chimpanzees have been shown to induce humoral and cellular immunity after intramuscular immunization and to decrease the viral load in plasma [1, 2, 3]. A fear of genetic vaccination, however, is that injection of naked DNA may induce anti-DNA antibodies, giving rise to autoimmune diseases such as systemic lupus erythematosus [4]. Phase I clinical studies using an HIV-1 gp160-coding DNA in HIV-1-infected asymptomatic individuals were performed in Philadelphia (USA) and Zurich (Switzerland). The recently published Philadelphia part of the study was designed as a dose-escalation study in which doses of 50, 100, 200 and 300 μg of DNA vaccine were investigated [5, 6]. Here, we report the part of the study performed in Zurich, where repetitive injections of the highest dose (4 times 400 μg of DNA administered at weeks 0, 4, 10 and 28) were applied and the follow-up period was prolonged up to 52 weeks. The results were similar to those of the Philadelphia study, though discrepant findings also were obtained.

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Materials and Methods

Study Subjects

Four asymptomatic HIV-1-infected but otherwise healthy subjects (2 males, 2 females; mean age 33±6 years) were selected for the trial. Inclusion criteria comprised a positive serum anti-HIV-1 antibody enzyme-linked immunosorbent assay (ELISA) confirmed by Western blot analysis, CD4+ lymphocyte counts ≥500/μl and normal hematologic values and serum chemistry. Exclusion criteria comprised the presence of HIV-related symptoms, antiretroviral therapy, a history of hepatitis B, C and HTLV-1 infection or the presence of anti-DNA autoimmune antibodies. The plasma HIV-1 concentration was monitored throughout the study but was not a subject of specific requirement.

Vaccine

The vaccine was a specially constructed DNA plasmid (APL400–003, developed and produced for the trial by Apollon, USA) formulated with bupivacaine, an amide-type anesthetic agent that increases skeletal myocyte metabolic activity and sub-

Table 1. CD4+ and CD8+ lymphocyte counts, HIV-1 viral load and anti-dsDNA antibodies in blood samples of four HIV-1-infected subjects before and after repetitive injection of anti-HIV-1 gp120 plasmid vaccine

Parameter	Patient no.	Baseline	Week 28	Week 52	Last follow-up[a]
CD4+ lymphocyte count/μl	1	700	693	715	667
	2	540	187[b]	836	688
	3	610	654	768	448
	4	1,110	1,135	1,171	1,401
CD8+ lymphocyte count/μl	1	830	819	1,367	888
	2	850	300	1,433	483
	3	1,200	1,334	1,589	1,512
	4	790	691	812	1,205
HIV-1 RNA/ml	1	374	261	198	1,265
	2	74,051	83,641[b]	372	135
	3	16,409	24,339	25,262	72,567
	4	415	671	<184	22,508
Anti-dsDNA antibodies (OD)[c]	1	0.128	0.110	0.108	n.d.
	2	0.107	0.102	0.094	n.d.
	3	0.125	0.116	0.143	n.d.
	4	0.126	0.108	0.118	n.d.

n.d., not done
[a] Last follow-up: patient 1, 160 weeks; patient 2, 163 weeks; patient 3, 181 weeks; patient 4, 157 weeks
[b] HAART initiated at week 28
[c] Optical density (OD) reading at 550 nm wavelength, cutoff value=0.286 (±0.018)
[d] Determinations as previously described [5]

sequent uptake of the plasmid [7, 8]. The plasmid DNA consisted of the puc18 backbone containing the human cytomegalovirus immediate-early promotor, *env* and *rev* genes from an HIV-1MN-like strain and a kanamycin resistance gene. The *env* gene encodes the precursor gp160 protein, which includes both the gp120 and gp41 mature *env* proteins, and the *rev* gene encodes the p19 regulatory protein. The *rev* gene product is expressed as a functional protein product, and the *env* gene has been modified to remove a segment of gp41 that is similar to normal major histocompatibility complex antigens.

Vaccination Schedule

Patients were inoculated by needle injection with a total amount of 400 μg of plasmid DNA, administered at four different sites as one injection of 100 μg in the deltoid muscle of each arm and in the quadricep muscle of each leg. Repetitive injections of 400 μg plasmid DNA were given at weeks 4, 10 and 24 after the first injection.

Laboratory Testing

Hematologic and clinical chemistry parameters as well as CD4+ and CD8+ lymphocyte counts were analyzed by standard methods (CD4+/CD8+ lymphocyte counts: flow cytometry). HIV-1 plasma concentrations were determined by the Roche Amplicor HIV-Monitor system (Roche Molecular Diagnostics, Switzerland) at a detection level of 200 RNA copies/ml. Serologic testing for anti-HIV-1, anti-HBs, anti-HCV and anti-HTLV-1 antibodies was done by microplate enzyme immunoassay and Western blot analysis. Anti-nuclear antibodies (ANA), anti-native DNA (anti-nDNA) and anti-double-stranded DNA (anti-dsDNA) antibodies were determined by immunofluorescence and microplate enzyme-linked immunosorbent assay (ELISA) systems. The baseline screening was done 2 weeks before the first injection of DNA. Follow-up analyses were done 28, 36 and 52 weeks after the first injection (4, 12 and 28 weeks after the fourth injection). The study ended at week 52. Later CD4+/CD8+ lymphocyte counts and HIV RNA determinations were done in the frame of the routine surveillance program for HIV-infected subjects. Antibodies against HIV *env* were determined at Apollon, using an ELISA as described previously [5].

Table 2. Anti-HIV gp120 antibodies in blood samples of four HIV-1-infected subjects before and after repetitive injection of anti-HIV-1 gp120 plasmid vaccine

Patient no.	Anti-gp120 antibodies (O.D.)[a]			
	Baseline	Week 3	Week 10	Week 20
1	0.709	0.828	0.773	0.792
2	0.425	0.345	0.411	0.410
3	0.421	0.380	0.390	0.386
4	0.274	0.193	0.245	0.249

[a] Optical density (OD) reading at 550 nm wavelength, cutoff value=0.286 (±0.018)

Results

The DNA vaccine was well tolerated and proved to be safe. Vaccine administration did not induce local or systemic reactions. No significant changes in hematologic or clinical chemistry parameters, including liver enzymes, were detected in any of the subjects at any time during the study. None of the subjects presented evidence of induction of anti-nDNA, ANA or anti-dsDNA autoimmune antibodies (Table 1).

No anti-plasmid DNA antibodies were detected by enzyme immunoassay analysis in any of the four subjects at any time during the study. Anti-gp120 and anti-gp160 antibody titers did not change significantly over time and especially did not increase in response to vaccination relative to the baseline value (Table 2).

The CD4+/CD8+ lymphocyte counts and plasma HIV-1 concentrations are given in Table 1. At the beginning of the study, the four subjects presented HIV RNA copy numbers ranging from below 500 (2 subjects) to 100,000 RNA copies/ml (1 subject). Viral loads did not change significantly over time in response to vaccination in any of the subjects. Subject no. 2, whose viral load remained high at approximately 80,000 RNA copies/ml despite four injections of the DNA vaccine, was started

on highly active antiretroviral therapy (HAART) at week 28. Subsequently, the HIV viral load dropped to levels below 200 RNA copies/ml, and the number of CD4+ cells increased to 1,433/μl.

Follow-Up Data

After more than 3 years, patient 1 is doing well clinically, with stable parameters and no antiretroviral therapy. Patient 2 decided to start HAART at week 28 and since then has done well. Patient 3, who is not receiving HAART, is doing well clinically but exhibits deteriorating immunological and virological parameters. Patient 4 is doing well clinically, with increasing viral load but stable CD4+ lymphocyte counts, and has not received HAART. All four individuals taken together do not present either improvement or faster progress of the disease. In summary, none of the four individuals experienced a therapeutic benefit from the DNA injections.

Discussion

The results of the clinical phase I studies performed in Philadelphia and Zurich using the identical anti-HIV-1 gp160 plasmid DNA construct in HIV-1-infected asymptomatic individuals showed similarities and discrepancies. In both centers the application of plasmid DNA vaccine was well tolerated and proved to be safe with respect to chemical and hematological safety parameters. No anti-plasmid DNA antibodies were detected among any of the combined 19 subjects. In addition, no significant effects on the surrogate markers for HIV infection such as viral load and CD4+/CD8+ lymphocyte counts in peripheral blood in response to HIV-1 DNA construct application were detected. These data in humans are in contrast to those in chimpanzees, in which a decrease of viral load in response to HIV DNA construct vaccination has been observed [1]. In contrast to the results seen in Philadelphia [5] and in chimpanzees [1], no significant increase in anti-gp120/gp160 antibodies was detected in the HIV-1-infected individuals investigated in Zurich. In the Philadelphia study, none of the five individuals in the 30 μg plasmid DNA group, only one of five in the 100 μg group and only two of five in the 300 μg group showed a significant increase in anti-HIV gp120 antibodies in response to plasmid DNA vaccine administration [5].

In Zurich, four injections of DNA at the highest dose of the whole study (400 μg of DNA per dose, administered at 4 different sites) were given, whereas in Philadelphia three injections of DNA escalating from 30 μg to 300 μg per dose (the whole dose given as 1 injection) were administered. Since in Zurich the accumulated dose was 1,600 μg of DNA, we were particularly interested in the induction of autoimmune antibodies, which was investigated by a supplementary ELISA test for anti-dsDNA antibody detection. In addition, the follow-up period in Zurich was longer than that in Philadelphia (52 vs. 36 weeks). Similar to the observations in the Philadelphia trial, no anti-DNA antibodies were detected, and none of the individuals developed signs of lupus erythematosus within the observation period. This is an important safety issue for future trials with DNA vaccines in humans, but the safety issues need to be clearly defined when including patients on antiretroviral therapy of other formulations or when using other doses of DNA vaccines. This study presents the results of the longest follow-up period ever published of individuals treated with a DNA construct.

Even though both trials were designed as phase I clinical trials, with special focus on safety, preliminary data suggest that vaccination with the present HIV-1 DNA construct did not show any virological or immunological efficacy, which is in contrast to findings in the chimpanzee model [1]. Recent results from our own laboratory in preclinical animal models have shown that DNA vaccines against influenza A virus are effective only against low virus concentrations [9]. This may explain the success of the chimpanzee study, in which slowly replicating virus was used [1], and may also explain the failure to achieve reduction of plasma virus concentrations in the human trials, in which viral loads are relatively high. In fact, vaccination of infected subjects receiving HAART seems to be a promising approach towards management of HIV-1 infection [10]. Novel trends clearly favor more complex vaccines combining HIV-1 DNA constructs with proteins for boosting [11, 12] or combining viral structural with viral regulatory genes and cytokine genes [13]. In the meantime, a vaccine trial with a DNA vaccine has also been performed in HIV-1-seronegative volunteers and has been shown to elicit humoral and cellular immune responses [14].

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References

1. Boyer GD, Ugen KE, Chattergoon M, Wang B, Shah A, Agadjanyan M, Bagarazzi ML, Javadian A, Carrano R, Coney L, Williams WV, Weiner DB: DNA vaccination as anti-human immunodeficiency virus immunotherapy in infected chimpanzees. Journal of Infectious Diseases (1997) 176:1501–1509
2. Bagarazzi ML, Boyer JD, Ugen KE, Javadian MA, Chattergoon M, Shah A, Bennett M, Ciccarelli R, Carrano R, Coney L, Weiner DB: Safety and immunogenicity of HIV-1 DNA constructs in chimpanzees. Vaccine (1998) 16:1836–1841
3. Wang B, Boyer J, Srikantan V, Ugen K, Agadjanian M, Merva M, Gilbert L, Dang K, McCallus D, Moelling K, Carrano R, Williams WV, Coney L, Weiner D: DNA inoculation induces cross clade anti-HIV-1 responses. In: Liu MA, Hillemann MR, Kurt R (eds): Annals of the New York Academy of Sciences: DNA vaccines: a new era in vaccinology. New York Academy of Sciences, New York (1995) pp. 186–197
4. Moens U, Sternes OM, Hey AW, Silsand Y, Traavik T, Johansen B, Rekvig OP: In vivo expression of a single viral DNA-binding protein generates systemic *Lupus erythematosus*-related autoimmunity to double-stranded DNA and histones. Proceedings of the National Academy of Sciences of the USA (1995) 92:12393–12397

5. MacGregor RR, Boyer JD, Ugen KE, Lacy KE, Gluckman SJ, Bagarazzi RB, Coney LR, Ginsberg RS, Weiner DB: First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. Journal of Infectious Diseases (1998) 178:92–100
6. Boyer JD, Chattergoon MA, Ugen KE, Shah A, Bennett M, Cohen A, Nyland S, Lacy KE, Bagarazzi ML, Higgins TJ, Baine Y, Cicarelli RB, Ginsberg RS, MacGregor RR, Weiner DB: Enhancement of cellular immune response in HIV-1 seropositive individuals: a DNA-based trial. Clinical Immunology (1999) 90:100–107
7. Danko I, Fritz JD, Jiao S, Hogan K, Latendresse JS, Wolff JA: Pharmacological enhancement of in vivo foreign gene expression in muscle. Gene Therapy (1994) 1:114–121
8. Coney L, Wang B, Ugen KE, Boyer J, McCallus C, Srikantan V, Agadjanyan M, Pachuk CJ, Herold K, Merva M, Gilbert L, Deng K, Moelling K, Newman M, Williams WV, Weiner DB: Facilitated DNA inoculation induces anti-HIV-1 immunity in vivo. Vaccine (1994) 12:1545–1550
9. Pavlovic J, Petrzilka D, van der Broek M, Moelling K: Protection against influenza A virus by DNA vaccination depends on the humoral response and dose of challenge virus. Vaccine (2002) (in press)
10. MacGregor RR, Boyer J, Ginsberg R, Lacy K, Higgins T, Baine Y, Ciccarelli R, Weiner D: Administration of a facilitated DNA plasmid vaccine containing HIV-1 *env/rev* and *gag/pol* genes to HIV-1 infected patients also receiving highly active therapy. Program and Abstracts of the 6th Conference on Retroviruses and Opportunistic Infections, Chicago (1999) Abstract no. 347
11. Okuda K, Xin KO, Tsuji T, Bukawa H, Tanaka S, Koff WC, Tani K, Okuda K, Honma K, Kawamoto S, Hamajima K, Fukushima J: DNA vaccination followed by macromolecular multicomponent peptide vaccination against HIV-1 induces strong antigen-specific immunity. Vaccine (1997) 15:1049–1056
12. Richmond JFL, Lu S, Santoro C, Weng J, Shiu-Lok Hu, Montefiori DC, Robinson HL: Studies of the neutralizing activity and avidity of anti-human immunodeficiency virus type 1 *env* antibody elicited by DNA priming and protein boosting. Journal of Virology (1998) 72:9092–9100
13. Kim J, Nottingham L, Vancott T, Manson K, Wyand M, Agadjanyan M, Boyer J, Ugen K, Weiner D: Multi-component DNA immunization provides significant immunity against immunodeficiency virus challenge. Program and Abstracts of the 6th Conference on Retroviruses and Opportunistic Infections, Chicago (1999) Abstract no. 41
14. Boyer J, MacGregor R, Ginsberg R, Cohen A, Vogt S, Schumann K, Baine Y, Ciccarelli R, Weiner D: Induction of cellular immune response in HIV-1 seronegative volunteers with a DNA vaccine. Program and Abstracts of the 6th Conference on Retroviruses and Opportunistic Infections, Chicago (1999) Abstract no. 44