Safety and Immunogenicity of a Canarypox-Vectored Human Immunodeficiency Virus Type 1 Vaccine with or without gp120: A Phase 2 Study in Higher- and Lower-Risk Volunteers

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Live attenuated viral vectors that express human immunodeficiency virus (HIV) antigens are being developed as potential vaccines to prevent HIV infection. The first phase 2 trial with a canarypox vector (vCP205, which expresses gp120, p55, and protease) was conducted in 435 volunteers with and without gp120 boosting, to expand the safety database and to compare the immunogenicity of the vector in volunteers who were at higher risk with that in volunteers at lower risk for HIV infection. Neutralizing antibodies to the MN strain were stimulated in 94% of volunteers given vCP205 plus gp120 and in 56% of volunteers given vCP205 alone. $CD8^+$ cytotoxic T lymphocyte cells developed at some time point in 33% of volunteers given vCP205, with or without gp120. Phase 3 field trials with these or similar vaccines are needed, to determine whether efficacy in preventing HIV infection or in slowing disease progression among vaccinees who become infected is associated with the level and types of immune responses that were induced by the vaccines in this study.

Developing effective vaccines to prevent human immunodeficiency virus type 1 (HIV-1) infection is the highest priority of the US Public Health Service, and effective vaccines are expected to have positive benefit on worldwide health and economy. Many live attenuated vaccines are among the most effective viral vaccines that have been developed. However, significant safety concerns over the use of live attenuated HIV-1 vaccines suggest that alternative approaches, which have the benefits of live attenuated vaccine without the risks, should be explored. Recombinant viral vectors expressing HIV-1 antigens are one approach to overcome the safety concerns of live attenuated vaccines [1–3]. Recombinant vectors are believed to behave similarly to live attenuated vaccines. The expression vector results in antigen processing through the major histo-

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The study protocol was reviewed and approved by the institutional review boards at each site. Informed consent was obtained from each volunteer in accordance with guidelines of the US Department of Health and Human Services and those of the authors' institutions.

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compatibility (MHC) class I pathway, which induces $CD8^+$ cytotoxic T lymphocytes (CTL). Furthermore, these $CD8^+$ CTL responses are believed to be important in controlling acute infection with HIV-1. Recent reports have correlated vigorous $CD8^+$ CTL responses with lower plasma viremia in HIV-infected persons [4].

Earlier studies have evaluated the safety and immunogenicity of vaccinia [5, 6] or canarypox [7] as a suitable vector for expressing HIV-1 antigens. In contrast to vaccinia, canarypox has the advantage of not being inhibited by the pre-existing immunity to vaccinia [7] that has been induced by routine immunization in the majority of adults born before 1970 in the United States. Boosting immune responses with recombinant gp160 or gp120 significantly improved the neutralizing antibody responses in volunteers who were given either vaccinia- [8–10] or canarypox virus–based vectors [11, 12].

The safety and immunogenicity of a canarypox vector expressing several HIV-1 antigens in persons at higher and lower risk for HIV infection were determined in this phase 2 study. The vector was constructed to express gp120 and core antigens; the core antigens are expressed and enzymatically cleaved to form a particle. This was done in an attempt to generate antigens that are presented by both MHC class I and MHC class II pathways. In anticipation of future trials to assess the efficacy of this or a closely related vector, we also sought to evaluate the potential contribution of a recombinant gp120 subunit given simultaneously to enhance antibody and helper T cell responses to HIV-1. Lower-risk subjects were included to bridge the data to previous phase 1 trials of the vaccines in case differences between responses of lower- and higher-risk subjects were detected. Higher-risk subjects were included as the likely target populations for future efficacy field trials. Also, these higher-risk persons potentially had been exposed to HIV antigens without infection, and the influence of this exposure on subsequent immune responses might enhance cellular or humoral immunity in response to vaccine. Therefore, we sought to compare immune responses to the vaccines among lower- and higher-risk subjects.

Important secondary questions were also addressed in this study. Assessment of the readiness of the research community to conduct larger trials of efficacy and evaluation of procedures that will be required for larger trials were goals of this study. Volunteers' behavior and resulting events that may affect the feasibility of future efficacy trials were described. Risk-taking behavior was monitored, and social risks that were experienced by participants were collected, along with their responses to these risks. The quality of blinding was assessed by monitoring the frequency of HIV antibody testing outside the study—that is, we assessed whether volunteers would get tested outside the study to unblind themselves.

Materials and Methods

Vaccines and Adjuvant

ALVAC vCP205. The recombinant canarypox virus, ALVAC-HIV vCP205 (Aventis Pasteur), expressed the products of several HIV-1 genes, including the p55 polyprotein expressed by the *gag* gene of HIV-1 LAI strain, a portion of the *pol* gene sufficient to express protease activity from HIV-1 LAI strain, gp120 expressed by a part of the *env* gene of HIV-1 MN strain, and the anchoring transmembrane region of gp41 of HIV-1 LAI strain. The *gag* gene, which codes for virus core antigens, is a relatively well-conserved gene. The *gag* gene encodes a polyprotein (55 kDa) that matures into the core proteins p24, p17, and p15, the last of which is then split into p9 and p6. This maturation process is catalyzed by the HIV protease encoded by a part of the *pol* gene and is the reason for the inclusion of the open-reading frame of the protease gene in the construction of ALVAC-HIV vCP205. Genotypic and phenotypic analyses of the ALVAC-HIV vCP205 recombinant were confirmed by nucleotide sequence and restriction analyses, as well as by Western blot and immune precipitation analyses. Derivation of the vector has been described elsewhere for similar vaccines [13, 14].

The recombinant vaccine virus was grown on specific pathogen–free chick embryo fibroblasts, and the vaccine was suspended in a solution of serum-free, antibiotic-free culture medium, containing virus stabilizers, and was lyophilized. Each 0.5 mL dose of the vaccine contained $10^{6.0}$ $TCID_{50}$ of virus.

HIV-1 SF-2 rgp120. Subunit vaccine was an intact glycosylated form of HIV-1 SF-2 rgp120 (Chiron Corporation) and was given as 0.5 mL doses containing 50 μg of antigen in MF59 adjuvant. The immunogen was derived through recombinant technology in CHO cells under the control of the cytomegalovirus immediate early–1 promoter. It was purified by ion exchange, hydrophobic interaction, and gel chromatography, as described elsewhere [15]. The immunogen was formulated in a vehicle composed of buffered saline.

Adjuvant. The adjuvant for the HIV-1 SF-2 rgp120 (MF59; Chiron Corporation) consisted of 0.5% polysorbate 80 (Tween 80 [Sigma] and polyoxyethylene sorbitan mono-oleate) and 0.5% sorbitan trioleate in a citrate buffer [15]. Squalene (5%), a metabolizable lipid, constituted the oil phase. Emulsification by high-pressure homogenization resulted in a physically stable emulsion with a mean droplet size of <300 nm.

Placebos. The placebo ALVAC (Aventis-Pasteur) was a mixture of 10 m*M* Tris-HCI buffer at pH 9.0, virus stabilizer, and freeze-drying medium. Saline was used as a placebo control for HIV-1 SF-2 rgp120.

Volunteers

This multicenter, double-blind, randomized phase 2 safety and immunogenicity trial was conducted at 6 AIDS Vaccine Evaluation Group (AVEG) and 8 HIV Network for Prevention Trials (HIVNET) sites sponsored by the National Institute of Allergy and Infectious Diseases. Healthy HIV-1–uninfected adults aged 18-60 years were recruited into this study. Risk status for HIV infection was assessed at entry by a series of questions designed to identify

Table 1. Vaccine schema and distribution of volunteers according to human immunodeficiency virus (HIV) vaccine and recruitment groups (AVEG or HIVNET).

Group	Total no. of volunteers	Clinical sites and risk groups: AVEG Lower	AVEG Higher	HIVNET, higher	Immunization schedule, by month[a]: 0	1	3	6
A	140	20	40	80	A + gp120	A + gp120	A + gp120	A + gp120
B	140	20	40	80	A + Sal	A + Sal	A + Sal	A + Sal
C	140	20	40	80	PA + Sal	PA + Sal	PA + Sal	PA + Sal
Total	420	60	120	240				

NOTE. AVEG, AIDS Vaccine Evaluation Group; HIVNET, HIV Network for Prevention Trials.

[a] A, ALVAC-HIV recombinant canarypox virus vaccine expressing HIV antigens (vCP205), $10^{6.0}$ $TCID_{50}$/dose (Aventis Pasteur); gp120, SF-2 rgp120 (50 μg) in MF59; PA, placebo ALVAC (Aventis Pasteur); Sal, saline placebo.

risk for HIV-1 infection. The collaborating clinical trials groups in this study, AVEG and HIVNET, used group-specified standard criteria for defining the risk categories.

The definition of the low-risk group follows that used in the AVEG phase 1 studies, since the rationale for including a low-risk group in the phase 2 study was to ensure that immune responses to the new lot and new dose of vCP205 were comparable to what has been seen in phase 1 studies. Briefly, low-risk subjects were persons with ≤2 sex partners presumed to be HIV negative in the past 6 months, no injection drug use, and no newly acquired sexually transmitted diseases in the past 6 months [16].

The higher-risk participants were individuals from groups who may be asked to participate in phase 3 efficacy trials, including gay or bisexual men, injection drug users of either sex, and higher-risk heterosexual women. Participants at HIVNET sites were eligible for the study if they met the eligibility criteria for the HIVNET Vaccine Preparedness Study [16]. In brief, higher-risk subjects in HIVNET were, for men, persons reporting anal intercourse with another man in the last year, vaginal or anal intercourse with an HIV-infected woman in the last year, or injection of illicit drugs in the last 6 months. For women, entry criteria included injection of illicit drugs within the last 6 months, having a current HIV-infected male partner or a partner who had sex with other men, having ≥5 male sex partners within the last year, or exchanging sex for money or drugs within the last year. AVEG volunteers were defined as higher risk if they met the HIVNET criteria for defining higher risk, with the exception that men who had sex with men and were in long-term mutually monogamous relationships with a presumed HIV-negative partner were considered lower risk.

After informed consent was obtained, a history and physical examination were done, and screening laboratory assessments were performed. Eligibility criteria included a normal complete blood cell count (white blood cell count, 3500–12,000 cells/mm^3; total lymphocyte count, ≥800 cells/mm^3; platelets, 125,000–550,000/mm^3; and differential within institutional normal limits); hematocrit ≥30% for women, ≥38% for men; alanine aminotransferase ≤3 times the institutional upper normal limit; creatinine ≤1.6 mg/dL; normal urine dipstick test results, including esterase and nitrite; negative ELISA for HIV within 8 weeks of immunization; and availability for follow-up for a planned duration of ≥24 months.

Exclusion criteria included a history of immunodeficiency, chronic illness, autoimmune disease, a psychiatric condition, receipt of live attenuated vaccines within 60 days of the study, receipt of blood products or immunoglobulin in the past 6 months, active syphilis or tuberculosis (TB; but volunteers with a positive purified protein derivative test result and a normal chest x-ray showing no evidence of TB, and not requiring isoniazid therapy, were eligible), immediate type hypersensitivity reaction to egg products or neomycin (used to prepare ALVAC vaccines), prior receipt of HIV-1 vaccines or receipt of placebo in a previous HIV vaccine trial, and, in women, pregnancy or lactation.

Vaccine Schedule, Risk Reduction Counseling, Self-Reported Risk-Taking Behavior, Benefits and Risks of Participation, and Monitoring for Unblinding

Volunteers received 2 injections each at times 0, 1, 3, and 6 months (table 1). vCP205 (groups A and B) or placebo ALVAC (group C) was given intramuscularly in the left arm, and rgp120 (group A) or saline (groups B and C) was given intramuscularly in the right arm. Volunteers were evaluated 1 day after each injection for systemic and local reactions, and self-reported data were collected for 4 days. Data on serious adverse events were collected for 2 years of study.

At the time of each vaccination and every 3 months during the 24 months of follow-up, volunteers were given risk reduction counseling. These times were also used for monitoring for intercurrent HIV infection, collection of risk-taking behavior data, and collection of data on events related to participation. These latter observations included volunteers' perceived benefits and social harms of participation in the study. To monitor for possible unblinding of the study, volunteers were asked if HIV antibody assays had been done outside the study and the reasons for testing.

Immunogenicity

Serum was obtained from all subjects before initial vaccination, 2 weeks after doses 3 and 4 (3- and 6-month injection times), and at month 12. Humoral immune responses to HIV-1 were assessed at those times, including binding antibody reactivity by Sanofi HIV-1/HIV-2 peptide EIA kit (Genetic Systems), HIV-1/-2 ELISA (Abbott Laboratories), Western blot (commercial test kits varied by location), and EIA to gp120 (SF-2), as described elsewhere [17]. Neutralizing activity to HIV-1 MN was determined among the subset of volunteers who were also assayed for CTL (see below),

Table 2. Demographic characteristics of the 435 volunteers enrolled and stratified into the indicated arms of the study of human immunodeficiency virus type 1 (HIV-1) recombinant canarypox virus vaccine expressing HIV-1 antigens (vCP205), with or without the recombinant gp120 SF2 strain: 2 vaccine regimens versus 1 placebo regimen.

	Risk stratum for HIV, by vaccine group						
	Higher risk			Lower risk			
Characteristic	Placebo (n = 125)	vCP205 (n = 125)	vCP205 + SF-2 (n = 125)	Placebo (n = 20)	vCP205 (n = 20)	vCP205 + SF-2 (n = 20)	All subjects (N = 435), no. (%)
Sex							
Female	29	24	27	10	7	10	107 (24.6)
Male	96	101	98	10	13	10	328 (75.4)
Race							
White, non-Hispanic	83	77	81	17	20	15	293 (67.4)
Black, non-Hispanic	23	23	29	2	0	4	81 (18.6)
Hispanic/Latino	16	15	13	1	0	1	46 (10.6)
Asian/Pacific Islander	2	3	1	0	0	0	6 (1.4)
American Indian/Alaskan Native	1	1	0	0	0	0	2 (0.5)
Other	0	6	1	0	0	0	7 (1.6)
Median age (range), years	36 (18–55)	36 (21–58)	35 (19–56)	38 (19–60)	35 (22–53)	37 (18–53)	36 (18-60)

as described elsewhere [18, 19]. Neutralization of primary isolates of HIV-1 was not assessed in this study; little neutralizing of primary isolates was detected in a phase 1 study [20].

A subset of volunteers had peripheral blood cells drawn at the time serum was drawn (as described above), to assess cellular immune responses to HIV-1. These assays included HIV-specific *env* and *gag* $CD8^+$ CTL activity. Ninety volunteers each in groups A and B and 30 volunteers in group C were randomized into the CTL substudy. To maintain blinding of the treatment assignments, acid citrate dextrose–anticoagulated blood was collected from all AVEG volunteers (180 individuals) and from all volunteers at the HIVNET trial sites participating in the CTL/Neutralization substudy (~90 volunteers per vaccine group and 30 placebo recipients). The peripheral blood mononuclear cells (PBMC) from the day of initial immunization were cryopreserved at the AVEG Central Immunology Laboratory for future studies, for quality control and assay comparisons. $CD8^+$ CTL were determined at the central laboratory, using fresh PBMC (K. Weinhold, Duke University), as described elsewhere [20]. Lymphocyte proliferation assays were performed on the 44 Saint Louis volunteers at the Saint Louis University AIDS Vaccine Evaluation Unit, using cryopreserved PBMC, to assess T cell memory for *gag* and *env* by stimulation with rp24 and rgp120 SF-2 antigens, using methods described elsewhere [21, 22].

Statistical Analysis

χ^2 tests were used to compare frequency distributions, with Fisher's exact test used for 2×2 tables. Wilcoxon tests were used to test for treatment differences in vaccine reactogenicity scores. Antibody responses were compared by repeated measures analysis of variance, and reciprocal antibody titers below the level of detection were assigned a value of half the lower limit (e.g., a reciprocal titer of <10 was expressed as 5). The proportion of subjects who were $CD8^+$ CTL positive were examined with adjustment for repeated testing, using generalized estimating equation (GEE) methods. Analysis was done using the SAS version 6.12 software package (SAS Institute).

Results

Volunteer Accrual and Demographic Data

The first volunteer was entered on 22 May 1997, and the last volunteer was entered on 22 January 1998; 435 volunteers (4% above target) were enrolled. Table 2 summarizes the demographics for the volunteers. Sixty-nine volunteers were considered to be higher risk due to drug abuse and 306 due to sexual risk factors. Among the higher-risk men, 77 gave a history of sex with an HIV-infected partner, 213 had sex with a partner of unknown HIV status, 12 had receptive anal sex with an HIV-infected partner, and 61 had receptive anal sex with a partner of unknown HIV status. Among higher-risk women, 6 reported unprotected vaginal sex with an HIV-infected partner.

Vaccination and Vaccine Safety

Four hundred thirty-five volunteers received the first immunization, 432 received the second, 427 received the third immunization, and 416 received the fourth immunization. Reasons for not receiving all vaccinations included intercurrent HIV infection (5 subjects; one between screening and dose 1, two before dose 3, and two before dose 4). Two volunteers died: one due to homicide and the other due to cocaine overdose with subdural haemorrhage; each had received 3 vaccinations. Three volunteers were incarcerated, one each after the first, second, and third vaccinations. Three volunteers were lost to follow-up, two after the second vaccination and one after 3 vaccinations. Four volunteers either moved (one after the second vaccination) or refused to continue further in the study (1 volunteer each after the first, second, and third vaccinations). Two volunteers had seizures believed to be unrelated to the vaccine but were not given additional vaccine. Another volunteer was unable to continue due to an unrelated adverse event (uterine leiomyomas) after 3 vaccinations.

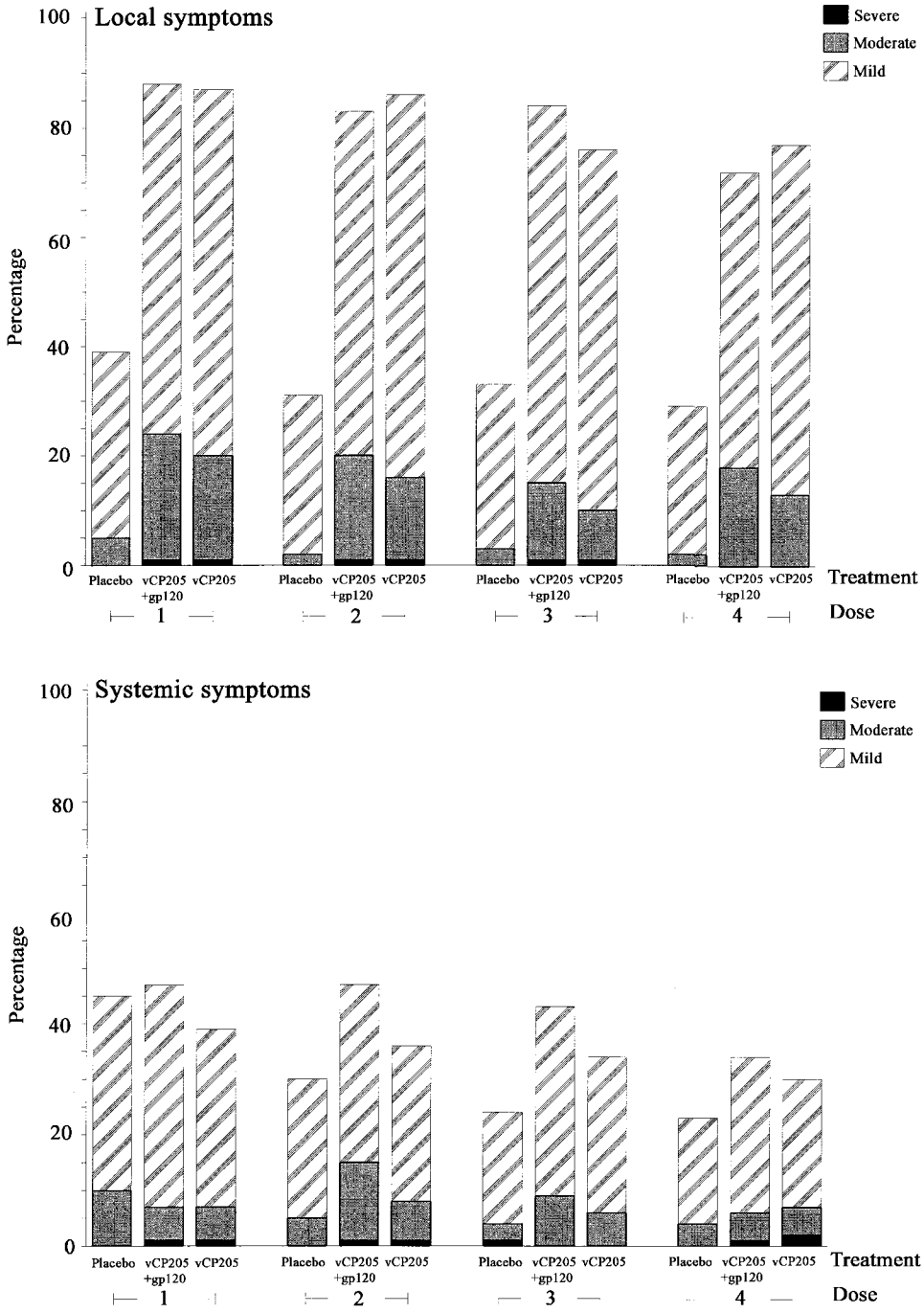

Figure 1. *Top,* Safety of recombinant canarypox vector expressing human immunodeficiency virus type 1 (HIV-1) antigens (vCP205), with or without recombinant envelope glycoprotein (gp120), in higher- and lower-risk persons. Bars indicate percentages of volunteers reporting pain or tenderness after the indicated dose of vaccine among those who received placebo, the combination vCP205 + gp120, or vCP205 alone. Adverse reactions were classified as mild *(hatched bars),* moderate *(gray bars),* or severe *(black bars),* as defined elsewhere [23]. *Bottom,* Percentages of subjects, divided into placebo group, combination vaccine group, and vCP205 alone, reporting systemic symptoms, defined as fever, nausea, malaise, myalgia, or headache, within 3 days of vaccination, after the indicated dose of vaccine.

The profiles of local and systemic reactions to the vaccines or placebo were not different between higher- and lower-risk subjects (data not shown). Moderate local pain or tenderness and erythema or induration at the injection site was significantly more common among subjects receiving one or both vaccines than among those receiving placebo (figure 1) [23]. Severe systemic events or high fever was uncommon. Severe systemic symptoms (severe malaise, myalgia, nausea, and/or headache) were reported by 9 volunteers (two with dose 1, two with dose 2, one with dose 3, four with dose 4); one was in the placebo group, five were in the vCP205 group and three were in the vCP205 + gp120 group. Severe local reactions (severe pain and/or tenderness) were reported by 6 volunteers (one reported severe pain and tenderness after both dose 1 and dose 2, one with dose 1, two with dose 2, and two with dose 3); none were in the placebo group, three were in the vCP205 group, and three were in the vCP205 + gp120 group. No volunteers had both severe systemic and severe local reactions, and volunteers were not excluded from additional vaccinations because of these reactions. Moderate systemic events (malaise, headache, or myalgia) were more likely to occur in vaccinated subjects than in subjects receiving placebo (figure 1). Among volunteers receiving vCP205 (left arm) + gp120 (right arm), there were no significant differences between left and right arms in reaction severity with respect to pain, tenderness, erythema, or induration (data not shown). However, among volunteers receiving vCP205 (left arm) + saline (right arm), there were significant differences between arms in reaction severity with respect to pain and tenderness (pain and tenderness greater in left arm than right arm in each case; $P = .001$), but there were no significant differences with respect to erythema or induration. Among the volunteers receiving ALVAC placebo + saline, there were no significant differences between left and right arms in reaction severity with respect to pain, tenderness, erythema, or induration. Overall, there was not an increase in local or systemic events with dose 4, compared with dose 1; rather, there was a trend toward decreased local and systemic events (figure 1).

Immunogenicity

Antibody responses. Subjects given vector plus gp120 developed significantly more neutralizing antibody than did subjects given vector alone (table 3). Vector alone induced low titers of neutralizing antibody in 56% of volunteers, and the addition of rgp120 SF-2 significantly increased the proportion of volunteers with neutralizing antibodies. However, compared with historic data from a previous study, in which vCP205 vector alone was given at times 0 and 1 month, followed by vCP205 + rgp120 SF-2 at 3 and 6 months (geometric mean neutralizing titer [GMT], 1:232; 95% confidence interval [CI], 127–427 to MN strain), the GMT of volunteers in the present study (1:48; 95% CI, 38–60 to MN strain), after simultaneous administration of vaccines vCP205 + rgp120 SF-2 at all 4 time points (0, 1, 3, and 6 months), was significantly lower.

Table 3. Development of human immunodeficiency virus type 1 (HIV-1) binding or neutralizing antibodies 2 weeks after the fourth vaccination with recombinant canarypox vector expressing HIV-1 antigens (vCP205), with or without recombinant gp120 antigen.

	Antibody assay			
Group	Neutralizing antibody to MN strain[a]	Abbott EIA[b]	Sanofi EIA[b]	Western blot (*env* bands)[b]
Placebo	0/43 (<10 [0])	2/114 (2)	0/21 (0)	0/11 (0)
vCP205 + saline	49/87[c] (14 [56])	84/115 (73)	0/37 (0)	4/26[d] (15)
vCP205 + gp120	81/87[c] (48 [93])	82/114 (72)	1/39 (3)	12/30[d] (40)

[a] Data are no. positive/total no. (geometric mean titer [%]).
[b] Data are no. reactive/total no. (%).
[c] $P < .001$.
[d] $P = .07$.

The majority of subjects vaccinated with either the vCP205 vector alone (73% positive) or vCP205 + gp120 subunit (72% positive) developed a reactive EIA by the Abbott HIV-1/-2 test by 2 weeks after dose 4 (table 3). The Sanofi EIA, an assay based on detecting antibody to a gp41 peptide not contained in the vaccine, infrequently (<2%) was positive. Western blotting detected *env* bands in 15% and 40% of subjects who received either vector alone or the vector and subunit, respectively ($P = .07$, Fisher's exact test). Binding antibody assay results to SF-2 vaccine antigen 2 weeks after the second, third, and fourth vaccinations were 0% at all times for placebo recipients, <5% at all times for vCP205 recipients, and 53%, 92% and 93%, respectively, for the vCP205 + gp120 vaccine group. There was no difference in vaccine immunogenicity between higher- and lower-risk subjects, as indicated by antibody to immunogen (data not shown).

Cellular immunity. CTL and lymphocyte proliferation assay results are summarized in table 4 and figure 2, respectively. At some time point during the study, 33% of vaccinated subjects developed $CD8^+$ CTL to *env* and/or *gag* HIV antigen. As expected, *env* or *gag* $CD8^+$ CTL activity was detected infrequently in volunteers receiving placebo. Only 2 positive responses were observed among placebo recipients, and these are believed to have been false positives, since HIV infection was not present in these subjects. The proportion of volunteers with $CD8^+$ CTL was not significantly different between the vector alone– (32 of 84 volunteers) and the vector + gp120–vaccinated group (24 of 86 volunteers; $P = .8$, GEE method). No difference in the frequency of CTL responses was found between higher- and lower-risk subjects (table 4; $P = .5$, GEE method). Addition of rgp120 to the vaccine regimen did not increase the $CD8^+$ CTL response to *env* in the vaccine group (table 4).

There was no correlation between development of binding antibody to p24 antigen and *gag* $CD8^+$ CTL results 2 weeks after the fourth vaccination in the subgroup of volunteers in whom both assays were conducted. Among 35 vaccinees who were antibody negative to p24, 11 had $CD8^+$ CTL to *gag,* and among 19 vaccinees with positive p24 antibodies, 5 had $CD8^+$ CTL to *gag* (*P* value not significant).

Table 4. Development of $CD8^+$ cytotoxic T lymphocytes (CTL) to *gag* or *env* in higher and lower risk volunteers according to vaccine groups given either recombinant canarypox vector (vCP205) expressing human immunodeficiency virus type 1 (HIV-1) antigens alone or with recombinant gp120 HIV-1 subunit antigens or placebo.

Vaccine group, risk	No. positive to indicated antigen/no. tested					
	After dose 3		After dose 4		After 12–18 months	
	At this time	Cumulative	At this time	Cumulative	At this time	Cumulative[a]
vCP205 + gp120						
Higher[b]	11/58	11/58	14/51	18/65	1/7	19/66
Lower	2/19	2/19	3/17	4/20	1/1	5/20
All	13/77	13/77	17/68	22/85	2/8	24/86
vCP205[c]						
Higher	12/56	12/56	18/55	24/64	5/15	27/66
Lower	1/16	1/16	4/15	4/18	1/2	5/18
All	13/72	13/72	22/70	28/82	6/17	32/84
Placebo[d]						
Higher	1/32	1/32	1/27	2/37	0/4	2/37
Lower	0/11	0/11	0/7	0/14	0/1	0/15
All	1/43	1/43	1/34	2/51	0/5	2/52

[a] rgp120 did not add to the *env* $CD8^+$ CTL responses. Cumulative $CD8^+$ CTL for *env* at the 12–18-month time for all subjects in the vCP205 + rgp120 group was 16/83, for the vCP205 group was 18/84, and for the placebo group was 1/51. The corresponding *gag* $CD8^+$ CTL responses were 15/86, 21/84, and 1/51, respectively.

[b] Higher-risk subjects did not have significantly different frequencies of $CD8^+$ CTL responses to either vaccine regimen ($P = .5$, generalized estimating equation [GEE] method).

[c] $CD8^+$ CTL responses were not significantly more frequent among subjects receiving vCP205 alone than among the group given vCP205 + rg120 ($P = .8$, GEE method).

[d] Both vaccine groups had significantly more positive $CD8^+$ CTL assays than had the placebo group ($P = .001$, GEE method).

Lymphocyte proliferation was detected in 8 (28%) of 29 volunteers after the fourth immunization with vCP205 alone or with rgp120 (figure 2). Proliferation to envelope glycoprotein was significantly more frequent among recipients of the combination rgp120 + vCP205 than among recipients of vCP205 alone ($P = .01$, Fisher's exact test).

Social Benefits and Risks of Participation

Volunteers were asked at 3-month intervals about the beneficial and negative consequences of study participation in their lives. Ninety-three percent of volunteers reported a beneficial impact of study participation at ⩾1 visits. The most commonly noted beneficial effects generally were a sense of helping to find a vaccine to prevent HIV infection, educational, or receiving risk reduction counseling. Volunteers were also asked whether study participation had a negative impact on 9 specific aspects of their lives (personal relationships, health insurance, life insurance, travel/immigration, military/other federal agencies, educational programs, employment, housing, and medical/dental treatment). They were given the opportunity to report any other negative impact not covered by the 9 categories. Overall, 106 volunteers (27%) reported ⩾1 negative impact within 24 months of entry. There was a statistically significant decrease in the number of reports between the first time (day 84 visit) participants were asked about negative impact and the second time this inquiry was made (day 168; $P < .001$). Reports of negative social impact were largely on personal relationships (117 reports) and less frequently involved health insurance (2 reports) or life insurance (5 reports) coverage, attitudes or behaviors of medical providers (3), loss of employment (2), or problems with a landlord (1).

Neither of the individuals reporting problems with health insurance was denied coverage; one was waiting for the study to conclude before applying for coverage, and the other had job-related changes in insurance unrelated to the trial. Two of the persons reporting life insurance problems were denied coverage, but only one denial of coverage was directly related to trial participation; in this situation, the participant had incorrectly reported to the insurance company that the participant was HIV positive, despite a negative test result. In both situations, staff at the sites have written letters to the insurance companies to clarify that participants were uninfected. Two volunteers reported being fired when their employers learned of their study participation. In both instances the vaccine study site staff wrote letters to the employers, but the volunteers did not want to pursue any further action and found other employment.

Potential Unblinding

Forty-seven volunteers had a total of 59 HIV antibody tests done outside the study; only one was specifically done to attempt to unblind the study. Reasons for HIV tests outside this study included participants' being in another research study ($n = 15$), medical admission or health examination ($n = 17$), insurance examination ($n = 7$), jail or detoxification center ($n = 9$), fear of HIV infection ($n = 4$), health care worker need-

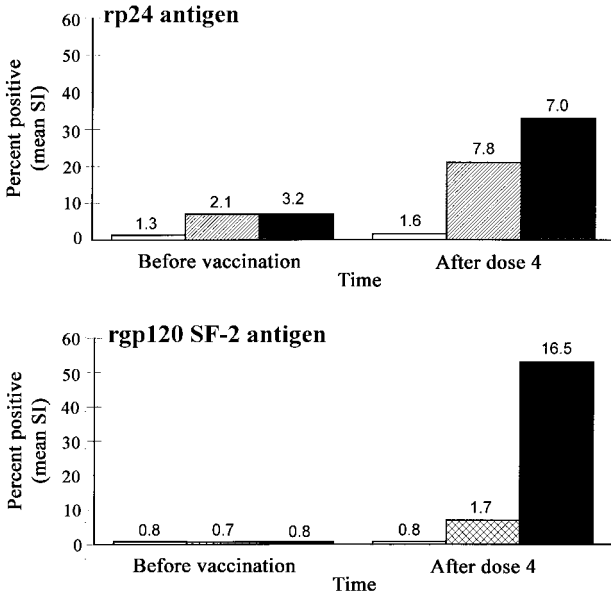

Figure 2. Lymphocyte proliferation responses to recombinant human immunodeficiency virus type 1 (HIV-1) antigens (rp24 antigen [*top panel*] or rgp120 SF-2 [*bottom panel*]) among 44 subjects given placebo *(open bars),* live recombinant canarypox vector (vCP205) expressing HIV-1 antigens *(hatched bars),* or vCP205 + rgp120 *(solid bars)*. Mean stimulation indices (SIs) are shown above the columns. A positive SI was defined as ⩾5. Significant differences in the proportion of subjects with positive lymphoproliferative responses to rgp120 SF-2 after dose 4 were present among the group receiving the combination vector vCP205 + rgp120 vaccination, compared with those receiving only vCP205 ($P = .01$, Fisher's exact test).

lestick injury ($n = 2$), selling blood ($n = 2$), other reasons ($n = 2$), and attempting to find out whether vaccine was received ($n = 1$).

Change in Risk Behavior over Time and HIV Infections among Volunteers

Selected self-reported risk-taking behaviors are summarized for year 1 of the study in figure 3. Overall, the level of self-reported risks decreased. Within each category there were some subjects who reported an increase in risk taking, but for each high-risk behavior there were as many or more subjects reporting a decrease in risky behavior, compared with those who reported an increase.

Intercurrent HIV infections and other severe adverse events are summarized in table 5. Fourteen HIV infections occurred among the volunteers during 2 years of follow-up. Of the 14 infections, 5, 2, and 2 infections occurred in the first year of study in the placebo, vCP205, and vCP205 + gp120 arms, respectively. Five infections occurred in the second year of follow-up in 1 placebo, 2 vCP205, and 2 vCP205 + gp120 vaccinees, respectively. One subject was found to be infected at the time of study. Excluding this case, the higher-risk group had 12 HIV infections in 837 follow-up years, for an attack rate of 1.43 per 100 years (95% CI, 0.74–2.50). One low-risk subject in 151 follow-up years was infected (attack rate, 0.66 per 100 years; 95% CI, 0.02–3.68). The single lower-risk subject was subsequently found to be in a mutually monogamous relationship with an HIV-infected partner who had been presumed to be HIV uninfected.

Discussion

The vaccines were safe and were generally well tolerated in this phase 2 study of the canarypox vector vCP205 with or without rgp120 subunit vaccine. Although either vaccine was more reactogenic than saline placebo, the local and systemic events were similar to those of other injected vaccines, and the antigens were not significantly different from each other in terms of reactogenicity. The live canarypox virus, therefore, seems suitable to pursue as a vector for antigen expression in attempts to derive the immunologic benefits of a replicating antigen without the risks of a live attenuated HIV vaccine.

One advantage of live replicating antigen for immunization is the induction of $CD8^+$ CTL in the peripheral blood. The rate of detection of $CD8^+$ CTL in other studies has been dependent on the number of assessments, since these effector cells are found at some times and not others after vaccination [24, 25]. Up to 5 time points were examined in the phase 1 study of vCP205, and in this large phase 2 study, 33% of subjects were positive at some time point when CTL were assessed on 3 occasions. No difference in response between higher- and lower-risk subjects was noted in our study. If some higher-risk subjects had previously been exposed to HIV antigens, this theoretical exposure did not measurably influence the $CD8^+$ CTL response. Although subunit vaccine did not increase $CD8^+$

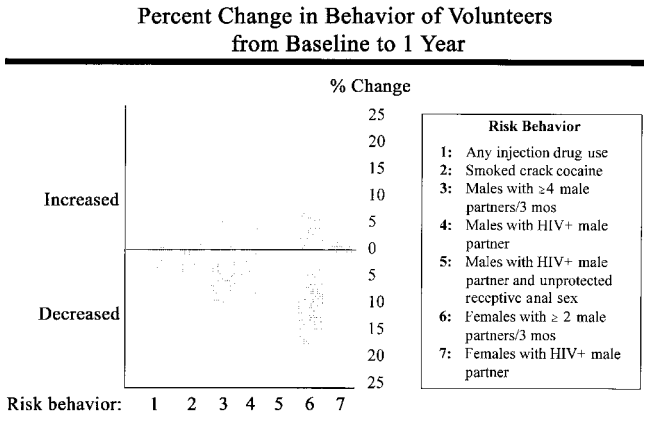

Figure 3. Percentages of higher-risk volunteers participating in this study of human immunodeficiency virus type 1 (HIV-1) vaccines with increases or decreases *(bars)* in self-reported high-risk behavior for 7 indicated behaviors. Data at baseline to 1 year are illustrated.

Table 5. Occurrence of intercurrent natural infection with human immunodeficiency virus type 1 (HIV-1) or death or life-threatening event other than HIV Infection, by study group.

Group *(n)*	HIV infection (intent to treat)	Follow-up years	Attack rate/ 100 person-years (95% CI)	HIV infection (after 4 doses)	Death or life-threatening event other than HIV[a]
Placebo (145)	6	327	1.84 (0.68–4.01)	3	6
vCP205 (145)	4	335	1.19 (0.32–3.05)	3	9
vCP205 + gp120 (145)	4	326	1.23 (0.33–3.15)	3	2
All (435)	14[b]	988	1.42 (0.78–2.39)	9	17

NOTE. Volunteers were previously vaccinated with recombinant canarypox vector expressing HIV-1 antigens (vCP205), with or without recombinant gp120 envelope subunit or placebo. CI, confidence interval.

[a] None of these were believed to be related to vaccination. They included hepatitis A (5), hepatitis B (1), homicide (1), arrhythmia (1), stroke (1), appendicitis (1), myocardial infarction (1), drug overdose (1), suicide attempt (3), and death (2).

[b] Of the 14 HIV infections, 5, 2, and 2 infections occurred in the first study year in the placebo, vCP205, and vCP205 + gp120 arms, respectively; the attack rate (95% CI) in year 1 for placebo was 3.54 (0.15–8.26), for vCP205 was 1.40 (0.17–5.06), for vCP205 + gp120 was 1.43 (0.17–5.16), and overall was 2.12 (0.97–4.03). In year 2 the attack rate (95% CI) for placebo was 0.74 (0.02–4.12), for vCP205 was 1.50 (0.18–5.42), for vCP205 + gp120 was 1.46 (0.18–5.27), and overall was 1.24 (0.40–2.89). One control subject was infected at the time of dose 1 of the study. Excluding this case, the higher-risk group had 12 infections in 837 follow-up years, for an attack rate of 1.43/100 years (95% CI, 0.74–2.50), while lower-risk subjects had 1 infection in 151 follow-up years (attack rate, 0.66/100 years; 95% CI, 0.02–3.68).

CTL responses, T cell memory for envelope glycoprotein was more frequently observed in the volunteers who received the SF-2 rgp120 subunit vaccine in addition to the vector vaccine.

The contribution of rgp120 to the immunogenicity of the vector and subunit vaccines was demonstrated by induction of antibodies and lymphocyte proliferative responses to *env.* Subjects receiving both vector and rgp120 had significantly more binding and neutralizing antibodies than those receiving only vector. Recently, other studies have noted that the magnitude of the neutralizing antibody response in this study was not as high as that in volunteers given these same vaccines on a different schedule [24, 25]. In another study of these same vaccines, when vaccines were given sequentially (i.e., doses of vCP205 were given alone at time 0 and 1 month, followed by vCP205 + rgp120 at 3 and 6 months), the GMT was 1:232 (95% CI, 127–427) to the MN strain [24, 25]. When vaccines were given simultaneously at times 0, 1, 3, and 6 months, the GMTs were 1:48 (95% CI, 38–60) in the present study and 1:45 (95% CI, 31–64) in a recently completed phase 1 study [25]. Vector alone gave low neutralizing antibodies (GMT, 1:14; 95% CI, 11–17). The mechanism by which simultaneous administration of rgp120 blunts the antibody response is not known, but design of future trials will include sequential administration of vaccines to improve the induction of antibodies. Vaccination with vector + rgp120 induced lymphocyte proliferative responses in 53% of subjects, which was significantly more frequent than induction of proliferative responses in subjects vaccinated with vector alone.

Commercial HIV antibody test kits that detected antibodies to a vaccine antigen became positive in the majority of vaccinated subjects. Many of these subjects also exhibited *env* antibody bands on Western blotting. Therefore, it was necessary to screen for intercurrent HIV infection, by using a test that measured antibody development to a nonvaccine antigen (gp41 in this instance) or an assay that detected nucleic acid. The Sanofi EIA, an assay based on detecting antibody to a peptide contained in gp41, proved to be a simple screening test, since only the transmembrane portion of gp41 was contained in the vector, and the subunit vaccine was limited to the gp120 portion of *env.* Confirmation of positive Sanofi EIA results was done by PCR and/or viral culture, to confirm intercurrent HIV infection in the 14 volunteers in whom this occurred.

Correlates of immune protection against HIV infection are not yet established. Only a phase 3 efficacy field trial can determine these parameters. Potential correlates of protection include neutralizing antibodies directed against laboratory strains, neutralizing antibodies directed against primary isolates, T cell memory responses such as lymphocyte proliferation, anti–HIV-1 $CD8^+$ CTL, immunologic priming for secondary antibody responses, or other immune parameters yet to be evaluated. The development of live viral vectors that induce a broad range of immune responses, including HIV-specific $CD8^+$ CTL, is the goal of an ongoing series of clinical trials funded by the National Institute of Allergy and Infectious Diseases jointly with partners in industry. A series of live attenuated canarypox vectors have been evaluated in phase 1 trials, and the most promising of those are moving forward into phase 2 clinical trials, such as the vector in the present report. One or more of these vaccines should be evaluated for safety and immunogenicity and efficacy in a phase 3 trial in higher-risk subjects. The occurrence of intercurrent HIV infection at a rate of 2.12% (95% CI, 0.47%–4.03%) in year 1 of follow-up suggests that entry criteria were appropriate: the

study was not powered to show differences in attack rates across groups, so no conclusions can be drawn from the attack rate in placebo subjects (year 1, 3.54%; 95% CI, 0.15%–8.26%) versus other groups. Further evaluation of these vaccines in efficacy field trials will be required to understand whether the immune responses observed in the present study are sufficient to protect against HIV infection. This phase 2 study lays the groundwork for these future efficacy field trials. Efficacy field trials are clearly feasible but will require scale-up to enroll large numbers of volunteers. The high rate of compliance by volunteers, including willingness to follow the vaccine and blood draw schedule, attend risk reduction counseling sessions, and not seek unblinding by obtaining HIV antibody tests outside of the study, confirm that efficacy field trials can be conducted to obtain the critical efficacy data on several different vaccine concepts. These trials are needed to guide the future development of effective HIV vaccines.

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