Recombinant gp160 as a therapeutic vaccine for HIV-infection: results of a large randomized, controlled trial

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Objectives: The primary objective of this study was to expand the safety and immunogenicity database of recombinant gp160 as a therapeutic vaccine in the treatment of HIV-infection. Preliminary efficacy data was also sought.

Design: This trial was a randomized, double-blind, placebo-controlled study. Two-hundred and eight volunteers, 96 therapy-naive with CD4 cell count $> 500 \times 10^6$/l (group A) and 112 with CD4 cell count of $200–500 \times 10^6$/l (group B, 51 out of 112 on treatment with one or two nucleoside analogues), received monthly injections of rgp160 IIIB vaccine or placebo for the first 6 months of the study; booster immunizations with rgp160 MN or placebo were given at times 15, 18, and 21 months.

Methods: Safety and immunogenicity data were obtained and measurements of CD4 cell count, plasma viral RNA, and proviral DNA were performed. Clinical outcome was recorded for the 24 months of study.

Results: The vaccine was safe and well tolerated. Despite the induction of new rgp160-specific lymphoproliferative responses and the presence of positive delayed type hypersensitivity skin tests to rgp160 at the end of the 24 month study, no effect on the natural history of HIV infection was detected. Within 24 months, AIDS-defining illnesses had occurred in 19 of the vaccinated volunteers and in 18 of the placebo recipients. Persons with higher plasma viral RNA levels and higher proviral DNA had a more rapid decline in CD4 cell count when compared to persons with lower values. Vaccine did not alter viral RNA or proviral DNA levels.

Conclusion: There was no clinical benefit to therapeutic immunizations with rgp160, despite the induction of new lymphoproliferative responses.

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Introduction

Therapeutic vaccination to reduce the consequences of HIV-infection or slow disease progression would be a valuable addition to antiviral chemotherapy which potentially could help to reduce the need for antiretroviral compounds. One group of vaccine candidates to be considered in this context are glycoproteins derived from the viral envelope. These compounds have been shown to induce protective immune responses that prevent or decrease the severity of infection in animal retroviral infections [1–7], and the HIV-1 envelope glycoproteins gp160 or gp120 have been tested as potential preventive vaccines in several phase I prospective vaccine studies (reviewed in [8,9]).

The recombinant glycoprotein (rgp160) vaccine used in the present study has been evaluated previously in phase I safety trials conducted in both HIV-1-seronegative and HIV-1-infected persons [10–14] and new immune responses, including lymphoproliferation, were induced.

Therefore, we undertook a phase II study to expand the safety and immunogenicity database and possibly obtain preliminary data on efficacy in two groups of infected persons, subjects with relatively intact immune systems (CD4 cell count $> 500 \times 10^6$ cells/l) and persons with moderate CD4 decline ($200–500 \times 10^6$ cells/l).

Materials and methods

Design and study groups

This study was conducted between 1993 and 1996 as a double-blind, placebo-controlled, and randomized study to evaluate the safety, immunogenicity and effects of a recombinant gp160 HIV-1 vaccine in infected persons and to obtain preliminary efficacy data after rgp160 vaccination, if possible. Sixteen clinical centres in eight European countries recruited persons aged > 18 years with HIV-1 infection. Volunteers were enrolled into two groups: group A consisted of male and female volunteers who were asymptomatic and had $> 500 \times 10^6$ CD4 T cells/l at entry, and who were not taking antiretroviral agents; group B consisted of male and female volunteers with CD4 cell counts of $200–500 \times 10^6$ cells/l and this group was allowed to take reverse transcriptase inhibitors. Antiretroviral therapy followed the accepted indications of 1993 and was based on the primary physician's decision. Prophylaxis for opportunistic infections was encouraged if patients developed AIDS-defining diseases or if the CD4 cell count decreased to $< 200 \times 10^6$ cells/l. Approval from the respective institutional review board was obtained by each participating centre. All volunteers gave written informed consent.

At study entry volunteers were randomized per clinic to vaccine or placebo in a 1 : 1 ratio. Computer generated random numbers determined the content of the identical syringes coded for each volunteer. Clinics had no access to codes except for emergencies. The study was monitored by an independent monitoring board, which reviewed quarterly data summaries prepared by the study statistician, who was the only trial team member with access to non-blinded data.

Patients received monthly injections of vaccine or placebo for the first 6 months. Follow-up visits were at months 7, 9 and 12 for clinical and laboratory assessment. A subsequent trial amendment provided for booster immunizations with vaccine or placebo at months 15, 18 and 21 with follow-up evaluations at months 16, 19, 22 and 24 (Fig. 1).

Statistical analysis

Descriptive statistics included medians and interquartile ranges for demographics and base line characteristics and means and SEM for virological and proliferative response data. Two-sided confidence intervals (CI) and t tests were used with significance defined as $P \leq 0.05$. CD4 cell count decline was analysed by medians and median differences of slopes of least square regression lines. The χ^2 test and Cohen's κ was used for dichotomous data. Time-to-event data were analysed by the Kaplan–Meier technique, log-rank and by Cox's proportional hazards model. Volunteers were analysed as

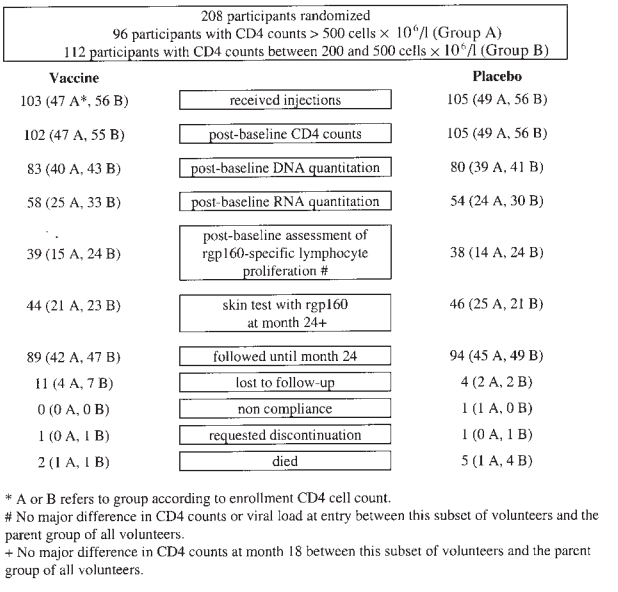

Fig. 1. Trial profile showing the immunization schedule and post-vaccination assessments of immunology, virology and compliance. Vaccinees received 100 μg rgp160 IIIB (months 1–6) and 100 μg rgp160 MN (months 15, 18, and 21) adjuvanted with deoxycholate and alum. Placebo recipients were given adjuvant alone.

randomized (intent-to-treat) except for two subjects (one in each arm) who were consistently switched by mistake.

Preparation and use of candidate vaccines, placebo and skin test reagent

Vaccine preparations, skin test reagents and placebo were provided by IMMUNO AG, Vienna, Austria. Production and purification of rgp160 IIIB and rgp160 MN were as described [15]. The vaccine was formulated at 50 μg/ml rgp160 with 0.25% deoxycholate, 0.20% aluminium hydroxide, 0.1% thiomersal in phosphate-buffered saline at pH 7.4. The placebo consisted of adjuvant and thiomersal at identical concentrations. The vaccine was divided into two portions and given by intramuscular injection of 1 ml into each arm (total dose 2 ml containing 100 μg antigen for vaccine recipients) with the exception of haemophiliacs who received subcutaneous injections. The rgp160 IIIB antigen was used for the first six vaccinations and the rgp160 MN antigen was given as the booster at 15, 18 and 21 months. Skin test reagent consisted of 10 μg or 100 μg rgp160 MN without adjuvant.

Clinical and laboratory evaluation

The protocol provided for two baseline CD4 cell count assessments of volunteers within 1 month before the first vaccination. Each volunteer had a physical examination, complete blood count, blood chemistry and urine analysis (and pregnancy test for women) at screening and prior to each vaccination and at each follow-up visit. CD3, CD4 and CD8 lymphocyte subset analyses (by two-colour flow cytometry, whole blood procedure) were performed at baseline and at months 4, 7, 12, 15, 18, 21 and 24 at each participating centre. After each immunization, volunteers were observed for at least 30 min with body temperature and any reactions recorded at the end of the period. In addition, patients were requested to record any events during the vaccination and follow-up periods of the study to include the following information: temperature twice daily for 7 days after each vaccination, any medication taken, days off work due to illness and hospitalization (in/out patient) with reason for it and duration.

Virology

A subset of volunteers had quantitative determination of HIV-1 RNA plasma level at baseline and at months 4, 7, 12 and 24, and proviral HIV-1 DNA quantified at baseline and at months 4, 7, 12 and 15 (Fig. 1). For quantification of HIV-1 RNA and DNA, quantitative competitive reverse transcription PCR or quantitative competitive PCR was used as described previously [16,17].

Lymphocyte proliferation and skin testing

Induction of T-cell memory was assessed in a subset of 77 volunteers (39 vaccine and 38 placebo). Tested were all volunteers from selected centres which could guarantee timely transport of blood to the core laboratories participating in the lymphocyte proliferation studies. There were no significant differences between volunteers with lymphocyte proliferation assay data and the parent group in terms of both baseline CD4 cell counts and baseline RNA levels.

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood according to standard procedures [18,19], washed and suspended in RPMI 1640 medium supplemented with 10% pooled, heat-inactivated (56°C, 30 min.) HIV-1 antibody-negative AB serum, antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin) and L-glutamine (2 mM) (complete medium). Quadruplicate cultures were then set up in flat-bottomed microtiter plates (1×10^5 PBMC/well in 0.2 ml complete medium) and the cells were incubated (37°C, CO_2 incubator) for 7 days in the presence or absence of rgp160 at a concentration of 1 μg/ml or an equivalent amount of mock antigen. Mock antigen contains the small amounts of vaccinia, cellular and medium proteins still present in rgp160 after purification and was used to control for possible proliferative responses to these contaminants. For the last 4 h of the incubation period ^{3}H-thymidine (1 μCi/well) was added to the cultures and the cells were then harvested onto glass fibre filters and incorporated radioactivity was measured in a β-counter.

A skin test for delayed type hypersensitivity was not available at the start of the study. After it became available it was performed according to a protocol amendment as an optional test at the end of the 24-month study in all patients who gave written informed consent. Based on previous information [20], two doses of rgp160 antigen, 10 μg and 100 μg, were used for skin testing. The vaccine antigen, rgp160 MN was dissolved in saline to give the appropriate concentration and 0.1 ml of each dose was adminstered simultaneously in each forearm. The skin test was considered positive if an induration of $\geq 5 \times 5$ mm was observed after 48 h.

Results

Enrolment and vaccination

Two-hundred and eight volunteers participated in this study; 96 volunteers had $> 500 \times 10^6$ CD4 cells/l at entry (group A) and 112 volunteers had $200–500 \times 10^6$ CD4 T cells/l at entry (group B). There were 25 women and 183 men in the study, and there was no significant difference in their distribution into vaccine or placebo group (Table 1). Similarly, there was no difference in the vaccine or placebo groups in baseline CD4 cell counts, baseline viral RNA copies/ml of plasma or baseline proviral DNA copies/10^5 cells. Fig. 1 summarizes the randomization, follow-up and

Table 1. Demographic characteristics of study participants at baseline.

Baseline characteristics	Group A		Group B		All
	Vaccine	Placebo	Vaccine	Placebo	
Demographic characteristics					
Sex (n)					
Male	40	44	47	52	183
Female	7	5	9	4	25
Age (years)					
Median	33	37	39	37	36
Range	21–59	23–64	22–69	19–61	19–69
Taking antiretroviral drugs (n)					
Yes	0	0	23	28	51
No	47	49	33	28	157
CD4 lymphocyte count ($\times 10^6$ cells/l)					
Median (n)	621 (47)	646 (49)	368 (56)	323 (56)	460 (208)
Interquartile range[a]	546–725	559–755	319–426	270–407	339–603
Median HIV-1 DNA copies per 10^5 cells (n)	13 (40)	5 (39)	16 (43)	29 (41)	14 (163)
Interquartile range	3–25	2–15	6–31	8–67	4–34
Median HIV-1 RNA copies per ml plasma (n)	60 000 (27)	10 000 (28)	81 000 (34)	64 000 (31)	59 000 (120)
Interquartile range	9000–109 000	2000–72 000	31 000–176 000	17 000–160 000	9000–135 000

[a]25th percentile to 75th percentile.

compliance. Of the 208 participants, 183 completed the 24 months of study; seven patients had died during this time. Compliance with immunizations was high; 94% received all six injections of rgp160 IIIB or placebo during the initial phase of the study and 76% of participants received the three booster injections of rgp160 MN at months 15, 18, and 21. Only 3.7% of scheduled injections were missed by the subjects.

Safety and adverse events

Pain at the injection site was common in both the vaccine and placebo recipients. Systemic complaints including fever, malaise, myalgia, arthralgia and headache were observed with equal frequency in the vaccine and placebo group. These symptoms were usually mild and of short duration. Among the seven patients who died during the study five had received placebo and two had received vaccine. The causes of death were one each of suicide, heart failure, trauma, liver failure with hepatitis B and C, intestinal infection and wasting, central nervous system toxoplasmosis, and unknown. No significant differences between vaccine or placebo groups were observed in the safety laboratory tests (data not shown).

Lymphocyte proliferation and skin tests

Fig. 2 shows lymphoproliferation in response to stimulation with the rgp160 IIIB, rgp160MN and the mock antigens. All but one of the vaccinated group A (14 out of 15) and 14 out of 24 vaccinated group B volunteers showed a positive lymphoproliferative response after rgp160 immunization (a positive response is defined as three or more rgp160-induced proliferative responses which are greater than three times the medium background but at least greater than 2000 cpm). The positive proliferative response was not limited to the homologous rgp160 IIIB (Fig. 2, upper panels), as rgp160 MN-specific lymphocyte proliferation was already present prior to boosting with MN subtype vaccine (Fig. 2, centre panels). The mock antigen preparation (Fig. 2, lower panels) gave a much lower lymphoproliferative response.

HIV-1 Env-induced lymphoproliferation data were confirmed by development of positive delayed type hypersensitivity skin reactions in both groups of vaccine recipients following subcutaneous application of a rgp160 MN preparation at month 24. The results of these skin tests are shown in Table 2. They indicate a significantly higher proportion of positive skin tests in the vaccination group. There also was a high degree of concordance (κ, 0.64; χ^2, 13.6, $P = 0.0004$) between a positive lymphoproliferative response and a positive skin test result (Table 3).

CD4 cell counts

Among all group A volunteers (with initial CD4 cell counts $> 500 \times 10^6$ cells/l) the CD4 cell count decreased with a median of 62×10^6 cells/l per year (95% CI, $47–91 \times 10^6$). Among the vaccine recipients in this group, CD4 cells decreased by 96×10^6 cells/l per year (95% CI, $57–107 \times 10^6$) and in subjects receiving placebo CD4 cells fell by 49×10^6 cells/l per year (95% CI, $13–76 \times 10^6$). In group B volunteers (with baseline CD4 cell counts of $200–500 \times 10^6$ CD4 cells/l) the CD4 cell count decreased by a median of 41×10^6 cells/l per year (95% CI, $25–56 \times 10^6$); the vaccine group had a median decline of 35×10^6 cells/l per year (95% CI, 20–63) and the placebo recipients had a median decline of 48×10^6 cells/l per year (95% CI, $17–74 \times 10^6$). In both groups of volunteers the difference between vaccine and placebo recipients was not statistically significant.

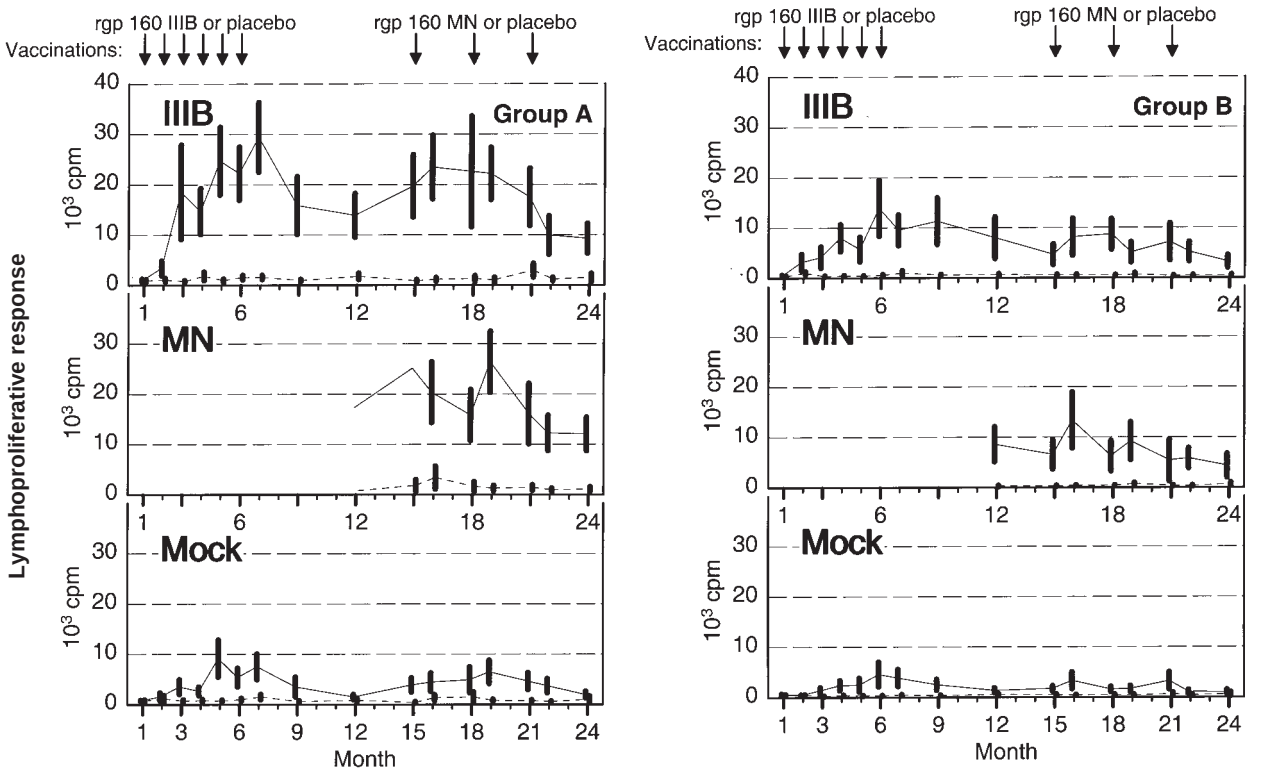

Fig. 2. Induction of lymphoproliferation to rgp160 IIIB (upper panels), rgp160 MN (centre panels) or mock antigen preparation (lower panels). Group A (CD4 cell count > 500×10^6 cells/l) and Group B (CD4 cell count 200–500×10^6 cells/l) were given vaccine (solid line) or placebo (broken line) at the indicated times (arrows). Values represent mean values ± standard error of the mean.

Table 2. Occurrence of positive delayed type hypersensitivity skin test (5 × 5 mm induration) with 10 μg of rgp160 MN vaccine preparation at the end of the 24 months of study.

	Group A		Group B		All	
Skin test response	Vaccine (n = 21)	Placebo (n = 25)	Vaccine (n = 23)	Placebo (n = 21)	Vaccine (n = 44)	Placebo (n = 46)
Positive	17*	5	17**	6	34***	11
Negative	4	20	6	15	10	35

*χ^2, 17.0, $P = 0.0001$; **χ^2, 9.0, $P = 0.0059$; ***χ^2=, 25.6, $P < 0.0001$.

Virology assessment

To assess the potential impact of vaccine on plasma viral RNA and proviral DNA, subjects were stratified at entry according to treatment with antiretroviral compounds (for number of subjects taking or not taking antiretrovirals see Table 1). As shown in Fig. 3, there was no significant change over time in either HIV RNA or proviral DNA copy numbers in volunteers taking or not taking antiretroviral compounds. Among the volunteers taking antiretroviral compounds, there was a trend towards increasing proviral DNA (and higher plasma RNA on one occasion) with the placebo group. However, this did not achieve statistical significance. Vaccination by itself did not increase viral RNA or DNA (assessed 4 weeks after administration).

Clinical course

Although this study was not designed as a phase III efficacy study, sufficient AIDS-defining events occurred in group B (CD4 cell count 200–500×10^6 cells/l) relative to group A (CD4 cell count > 500×10^6 cells/l) to be significant after 24 months (data not shown).

Table 3. Numbers of volunteers with both lymphocyte proliferation assay and skin tests performed.

Proliferative response to gp160	Skin test	
	Positive	Negative
Yes	14	2
No	4	13

κ, 0.64. χ^2, 13.6, $P = 0.0004$

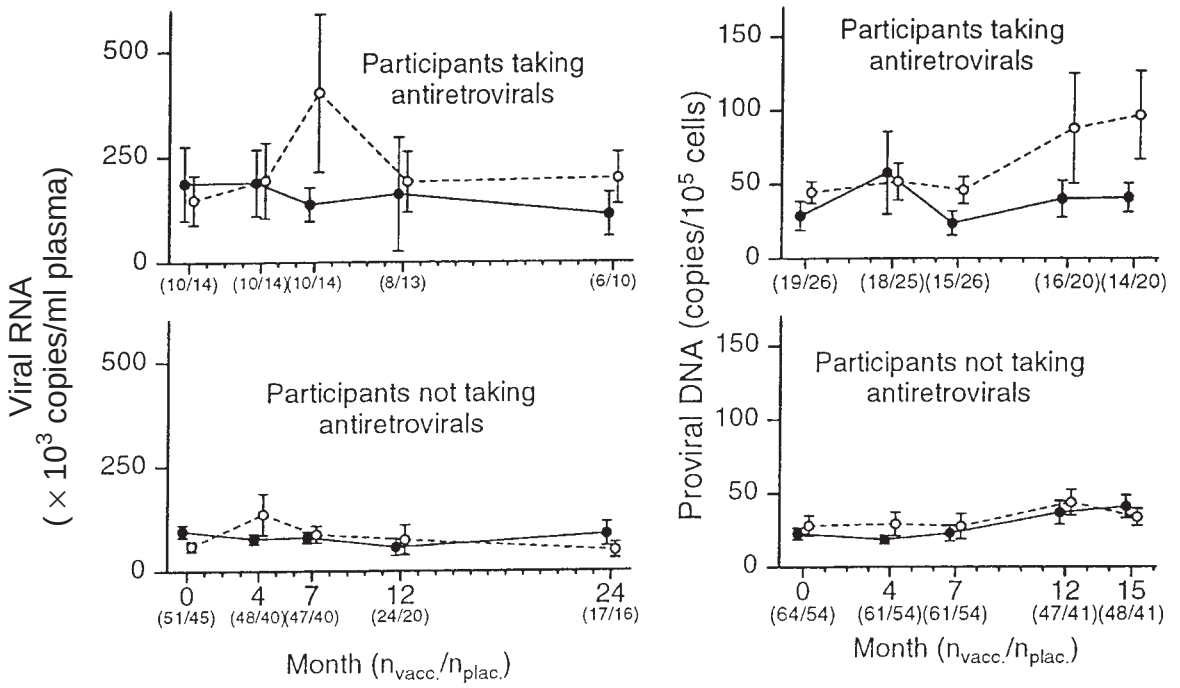

Fig. 3. Assessment of viral RNA in plasma and proviral DNA during the study. Participants were stratified according to antiretroviral treatment and vaccine (●) or placebo (○).

However, no impact of vaccine could be determined in this time, nor was there a trend. Among the 103 vaccinated volunteers, there were 19 with one or more AIDS-defining events and among the 105 placebo recipients, there were 18 with one or more AIDS-defining events (χ^2, 0.06, $P = 0.86$). Results of time-to-event analyses were similar (data not shown).

Similarly, when stratified by baseline CD4 cell count, no effect of vaccine on CD4 cell count decline was observed. Within 24 months 132 of 208 subjects had experienced a decline in CD4 cell count of ≥ 33% from baseline or to $< 200 \times 10^6$ cells/l. Among group A, 58 (32 vaccine versus 26 placebo), and in group B 74 (34 vaccine versus 40 placebo) developed this immunologic deterioration. There was no statistically significant difference between vaccine and placebo recipients in the probability of CD4 decline in either group A or group B (Fig. 4a). When these subjects were stratified according to viral RNA or proviral DNA at baseline, a significant difference in probability of CD4 decline was observed. However, no significant difference between vaccine and placebo recipients was found in these analyses (Fig. 4b and 4c).

Discussion

Despite induction of new lymphoproliferative responses by vaccination with rgp160 IIIB and rgp160 MN, no clinical benefit was found in this trial. This was true for subjects with relatively intact immune systems as shown by baseline CD4 cell counts of $> 500 \times 10^6$ CD4 cells/l, as well as subjects with moderate CD4 cell decline ($200–500 \times 10^6$ CD4 cells/l) at study entry. The results of the trial also imply, therefore, that CD4 cell count as the only marker for patient selection is not sufficient in future vaccination trials as evidenced by a comparatively high plasma viral RNA and by a considerable proportion of patients progressing to AIDS even in group A. Clinical outcome was predicted by either viral RNA or proviral DNA at baseline as has been shown by others [21–25]. When subjects were stratified into high or low plasma RNA or proviral DNA baseline levels, vaccine had no effect on CD4 cell count (see Fig. 4) or clinical events (data not shown). Although there was no benefit from vaccine, neither was there an adverse effect. The vaccine was safe and well tolerated, and use of vaccine was not associated with any change in CD4 cell decline, viral RNA in plasma or proviral DNA.

Previous studies of vaccines in infected persons have included phase I studies of this and other gp160 preparations [14,26–31], gp120 [32], HIV-1 core proteins [33–35], whole inactivated HIV-1 [36,37], live vector vaccine including canarypox [38] and polynucleotide vaccine [39]. Larger studies to evaluate some of these vaccines for efficacy are currently ongoing, but to date the results on efficacy have not been reported. Although the present trial was not designed as a phase III efficacy trial, the long duration of follow-up (2 years) in both vaccine and placebo groups resulted in

sufficient AIDS-defining events to suggest that this type of vaccine was not highly efficacious.

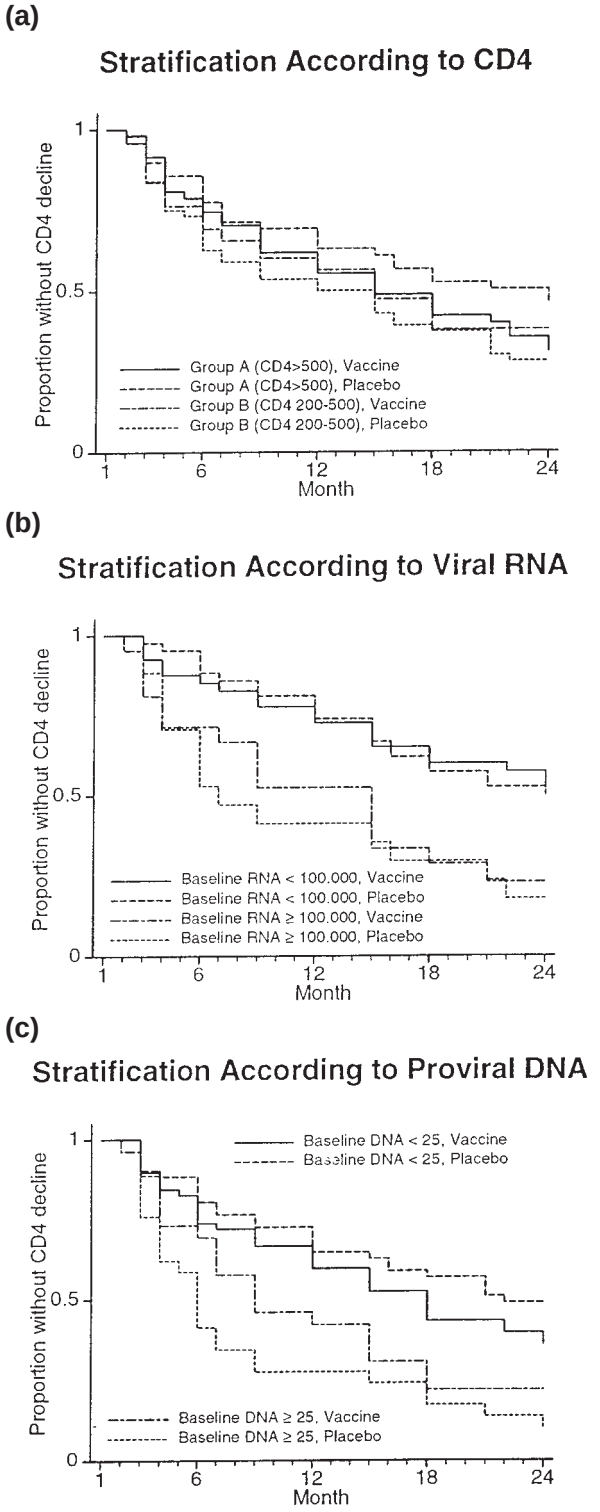

Fig. 4. Probability not to suffer from CD4 decline in vaccine and placebo recipients stratified according to CD4 cell counts, viral RNA in plasma and proviral DNA. CD4 decline was defined as > 33% decline from baseline or a drop to < 200×10^6 CD4-positive cells/l.

New immune responses to HIV antigens among infected persons who have received an HIV vaccine have included the induction of lymphocyte proliferative responses to HIV Env [27–31], as found in the present study. Although lymphocyte proliferation responses to Env would be an expected response to HIV-1-infection, this is not usually the case. Vaccination clearly is adding this type of immune response to the infecting agent. In previous studies, gp160 has been a much more powerful inducer of lymphoproliferation than has gp120 [8] and the vaccine used in this study was shown to have significant immunogenicity in uninfected persons, particularly with regard to lymphocyte proliferation [10,11,13]. Recent observations suggest that HIV-1-specific lymphoproliferative responses develop after treatment of acutely infected persons with potent antiretroviral drugs [40]. This has lead to speculation on the importance of HIV-1-specific T helper cell responses in controlling HIV infection. However, despite these immune responses that were induced in our study, the clinical outcome observed to date does not support this hypothesis.

Identification of immune parameters that correlate with slowing disease progression may refocus approaches on therapeutic vaccine in HIV. High levels of neutralizing antibodies, high frequency cytotoxic T-cell precursors, or other parameters may emerge as correlates of slow disease progression. Therapeutic vaccine to induce one or more of these parameters in conjunction with chemotherapy may provide significant immune control over the virus. Future trials should examine the effect of vaccine on persons who were vigorously treated with chemotherapy and responded with reduction in viral load.

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