Immunization with Recombinant p17/p24:Ty Virus-like Particles in Human Immunodeficiency Virus–Infected Persons

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In studies of the natural history of human immunodeficiency virus type 1 (HIV-1) infection, it has been repeatedly shown that higher-titer antibody responses to the HIV gag p24 protein correlate with less rapid disease progression. In HIV-negative persons, immunization with HIV-1 p17/p24:Ty virus-like particles (p24-VLP) induced humoral and cellular immune responses to p24. This construct was therefore studied as a potential immunotherapeutic agent with the objective of augmenting the immune response to p24 in a double-blind placebo-controlled trial involving 74 p24 antibody–positive, asymptomatic HIV-1–infected subjects with CD4 cell counts >350/mm^3. Immunization with p24-VLP was generally well tolerated. Immunization with p24-VLP did not increase p24 antibody levels and had no effect on CD4 cell counts or virus load. The failure to increase p24 antibody titers cannot entirely be explained by the subjects' immunodeficiency because most generated an antibody response to Ty, a yeast component of the immunogen.

In 1987, Salk and others hypothesized that immunization with human immunodeficiency virus type 1 (HIV-1) antigens could slow HIV-1 disease by augmentation of the immunity against HIV-1 [1]. In the natural course of HIV-1 infection, initial p24 antibody levels correlate with the AIDS-free interval, and the absence or decline of p24 antibody levels is related to more rapid disease progression [2–7]. However, the role of anti-p24 immunity in the pathogenesis of HIV infection is unclear. Immunization with HIV-1 p17/p24:Ty virus-like particles (p24-VLP) has been shown to induce p24 antibodies and cellular immunity to HIV-1 gag p17 and p24 components in HIV-seronegative persons [8].

We evaluated the safety of p24-VLP immunotherapy and tested its effect on p24 antibody titers, CD4 cell counts, and the number of HIV-1 RNA copies per milliliter (/mL) in asymptomatic HIV-1–infected subjects with CD4 cell counts >350/mm^3.

Materials and Methods

Vaccine. p24-VLP contain a part of the HIV-1$_{IIIB}$ gag sequence (codons 100–308), which comprises amino acids from the carboxy-terminal of gag p17 and from the amino-terminal of gag p24. Particles were produced by expressing a TYA:p17/p24 fusion gene in yeast [8]. The purified protein was adjuvanted to aluminum hydroxide at a ratio of 2:1; the placebo vaccine consisted of aluminum hydroxide only in PBS.

Subjects. Men and women, ages 18–60 years, were eligible for enrollment if they had an asymptomatic HIV-1 infection (CDC stage II/III), a p24 antibody titer >100, and a CD4 cell count of >350/mm^3 on each of the last three occasions (two of these measurements had to be taken before the first immunization) with a range (i.e., largest to smallest) <180/mm^3 or a coefficient of variation <25%.

Participants were ineligible if they had a relevant disease other than HIV-1 infection. Women of childbearing potential had to have a negative pregnancy test on screening and were required to use adequate birth control measures. Participants were not to have received antiretroviral therapy within the last 6 months or prophy-

Received 11 March 1996; revised 7 June 1996.

Presented in part: Conference on Advances in AIDS Vaccine Development: eighth annual meeting of the National Cooperative Vaccine Development Groups for AIDS, Bethesda, Maryland, February 1996 (poster 1).

Informed consent was obtained from all participants enrolled in this study. The project was reviewed and approved by the ethics committees of the five participating centers. This study was performed in compliance with regulatory requirements of the United Kingdom, Netherlands, and Belgium.

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The Journal of Infectious Diseases 1996;174:862–6

0022–1899/96/7404–0029$01.00

laxis for opportunistic infections or treatment with immunosuppressive agents at any time prior to the study. Subjects who were p24 antigen–positive were excluded, as were current intravenous drug users.

Experimental design. Subjects were assigned treatments in a double-blind fashion according to a minimization procedure. Doses of 100, 500, or 1000 μg of p24-VLP or placebo were administered intramuscularly in volumes of 0.5 mL into the deltoid muscle of the nondominant arm at weeks 0, 4, 8, 12, 16, and 20. The minimization procedure was used to balance treatment arms across baseline anti-p24 titers and CD4 cell counts, sex, and the five study sites [9].

Clinical evaluation. The injection site, blood pressure, heart rate, and temperature were monitored before and after each immunization. A physical examination was done at screening and every 12 weeks thereafter. Subjects were asked at each visit if any adverse events had occurred or ceased. Deterioration of any concurrent disease was considered to be an adverse event. The definition of the stage of HIV disease was based on the 1987 CDC classification.

Biochemistry, hematology, and urinalysis. Blood chemistry analyses, complete blood cell counts, and urinalyses were done regularly.

Immunogenicity. p24, p17, and Ty antibody measurements were done with an ELISA [8]. Results were expressed as the reciprocal end-point titers of an individual test serum. A ≥4-fold increase antibody titer from the week 0 value was considered a significant response.

Laboratory markers for disease progression. CD4 cells were counted in samples obtained between 0700 and 1200 h A.M. on 2 separate days during the screening period and in weeks 0, 4, 8, 12, 16, 20, 24, 36, 42, 46, and 48.

HIV-1 genomic RNA isolated from serum was quantified in a noncompetitive single-tube reverse transcriptase–polymerase chain reaction assay (Roche Biomedical Laboratories, Research Triangle Park, NC) [10]. Peripheral blood mononuclear cells were cocultivated with MT-2 cells to detect the presence of syncytium-inducing variants [11].

Statistical methods. Data were analyzed using the SAS software package (SAS Institute, Cary, NC) with analysis of variance (ANOVA) techniques. Differences in changes from baseline were compared between the immunized groups.

Results

Between March 1993 and March 1994, 74 subjects entered the study at five sites: Municipal Health Service (n = 21), University College London Medical School (n = 20), Institute of Tropical Disease (n = 15), St. Mary's Hospital (n = 10), and Royal Sussex County Hospital (n = 8). Sex, race, baseline mean CD4 cell counts, and screening p24 antibody titers in the treatment groups were well balanced (table 1). Of 74 participants, 5 (7%) did not receive all six immunizations; for 2 subjects this was due to adverse events (both received 500 μg of p24-VLP), 1 died (100 μg of p24-VLP), 1 withdrew because of clinical deterioration without progression in CDC group (100 μg of p24-VLP), and 1 simply missed the fifth immunization (100 μg of p24-VLP). Sixty-three participants (85%) completed the assessment at week 48.

Safety and tolerance. No consistent or important changes were observed in hematologic and biochemical parameters, blood pressure, or heart rate. Minor postimmunization events (temperature, local or systemic reactions) were experienced by all 74 patients and occurred to a similar extent in placebo and p24-VLP–treated subjects.

A 48-year-old participant with a long history of poorly controlled hypertension died suddenly 8 days after the second immunization (100 μg of p24-VLP). The cause of death was suspected to be cardiovascular. Local swelling, itching, and redness occurred in 1 subject (500 μg of p24-VLP) just after his fifth immunization; he had experienced a mild reaction after his fourth immunization. Another subject had a generalized urticarial rash within 2 h of his fifth immunization (500 μg of p24-VLP). This subject had had a swollen arm after the fourth immunization. Both subjects were withdrawn from immunizations but were followed.

Immunogenicity. Fourfold increases in p24 antibody levels occurred in a similar number of participants across all 4 treatment groups (table 2). Some of these increases occurred within the screening period (data not shown). Fourfold increases in p24 antibody titer did not appear to be related to CD4 cell count or p24 antibody titer at baseline.

Table 1. Sex, race, mean CD4 cell counts (weeks −4, −2, 0), p24-antibody titers, and biologic virus phenotype (week 0) of 74 HIV-1–seropositive participants in a dose-ranging study of HIV-1 recombinant p17/p24:Ty virus-like particles (p24-VLP).

		Race		CD4 cell counts/mm^3		p24 titers		Phenotype
	Men/women	White	Other	351–550	>550	101–10,000	>10,000	SI/NSI
Placebo	18/1	17	2	9	10	8	11	0/19
p24-VLP								
100 μg	16/2	16	2	8	10	8	10	1/17
500 μg	16/3	17	2	9	10	7	12	1/18
1000 μg	16/2	15	3	10	8	7	11	2/14
Total	66/8	65	9	36	38	30	44	4/68

NOTE. SI, syncytium-inducing; NSI, non-SI.

Table 2. Numbers of patients with a 4-fold increase in antibody titer (p24, p17, and Ty) at at least 1 point of time after immunization and split according to mean baseline CD4 cell count and p24-antibody titer.

	p24-VLP				
	100 μg	500 μg	1000 μg	Placebo	Total
p24 antibodies					
Total	2/18	4/19	5/18	5/19	16/74
Mean baseline CD4 cells					
350–550/mm^3	1/8	1/9	3/10	2/9	7/36
>550/mm^3	1/10	3/10	2/8	3/10	9/38
p24 antibodies (week 0)					
100–10,000	1/8	0/7	4/7	2/8	7/29
>10,000	1/10	4/12	1/11	3/11	9/44
p17 antibodies					
Total	10/18	7/19	3/18	4/19	24/74
Mean baseline CD4 cells					
350–550/mm^3	3/8	4/9	1/10	3/9	11/36
>550/mm^3	7/10	3/10	2/8	1/10	13/38
p24 antibodies (week 0)					
100–10,000	3/8	2/7	1/7	2/8	8/29
>10,000	7/10	5/12	2/11	2/11	16/44
Ty antibodies					
Total	8/18	14/19	17/18	1/19	40/74
Mean baseline CD4 cells					
350–550/mm^3	3/8	5/9	9/10	1/9	18/36
>550/mm^3	5/10	9/10	8/8	0/10	22/38
p24 antibodies (week 0)					
100–10,000	3/8	5/7	7/7	0/8	15/29
>10,000	5/10	9/12	10/11	1/11	25/44

NOTE. p24-VLP, HIV-1 recombinant p17/p24:Ty virus-like particles.

Changes in p17 antibodies were also not related to immunization with p24-VLP. However, responses to the yeast Ty antigen appeared to be related to the dosage of study drug and to the baseline CD4 cell count of the participants. In the 1000-μg group, all subjects but 1 generated an antibody response to Ty.

Clinical and laboratory markers for disease progression. A limited number of patients progressed to CDC group IV in the 48-week study. Five patients progressed from group II/III to group IVc2 (100 μg of p24-VLP, $n = 2$; 500 μg of p24-VLP, $n = 1$; 1000 μg of p24-VLP, $n = 2$), and 1 progressed to AIDS (1000 μg of p24-VLP). All progressions occurred in patients treated with p24-VLP, but there were no significant differences between the placebo group (0/19) and the group treated with p24-VLP (6/55) ($P = .16$).

Repeated immunization with p24-VLP did not induce significant differences in CD4 cell counts between the groups, neither in the short term, 4 weeks after immunization (ANOVA, $P = .582$), nor during the 48-week study period (ANOVA, $P = .656$).

The CD4 cell counts/mm^3 at baseline (mean of weeks −4, −2, and 0) and at the end of the study (mean of weeks 42, 46, and 48) were, respectively, 581 and 518 in the placebo group, 577 and 517 in the group receiving 100 μg of p24-VLP, 607 and 508 in the group receiving 500 μg of p24-VLP, and 565 and 472 in the group receiving 1000 μg of p24-VLP.

The number of HIV-1 RNA copies/mL was measured in a limited number of patients randomly selected from the placebo group ($n = 4$) and the group receiving 1000 μg of p24-VLP ($n = 10$). In the placebo group, a slight increase was present 4 weeks after the first immunization, with a similar level found 4 weeks after the last immunization (HIV-1 RNA in copies/mL $\log_{10}$: week 0 = 3.95, week 4 = 4.18, and week 24 = 4.31); in the group receiving 1000 μg of p24-VLP, a minimal decrease was observed (HIV-1 RNA in copies/mL $\log_{10}$: week 0 = 4.46, week 4 = 4.34, and week 24 = 4.38). These changes were not statistically significant ($P = .233$).

Discussion

The aim of this study was to test the safety and tolerance of p24-VLP immunization in asymptomatic HIV-1–infected persons and to examine changes in p24 antibody titers, CD4 cell count, and the number of HIV-1 RNA copies/mL.

In general, immunizations with p24-VLP were well tolerated. However, 2 subjects had type I hypersensitivity–like reac-

tions after the fifth immunization. Whether these reactions were indeed IgE-mediated and induced by a yeast, HIV-1, or another vaccine component cannot be ascertained from this study. One participant died during the study, but the cause of death was not related to administration of the study preparation.

p24-VLP did not increase p24 or p17 antibody levels in HIV-infected persons who had been selected for their ability to produce p24 antibodies prior to immunization. Although p24 constitutes only a third of the p24-VLP construct, the failure to increase p24 antibody titers cannot be due to a complete lack of antigenicity of p24-VLP or entirely to the immunodeficiency of the recipients. The antigenic properties of p24-VLP in doses of 100 and 500 μg have been demonstrated by the appearance of cellular and humoral immunity to HIV-1 gag p24 components in HIV-seronegative persons [8]. Of 9 HIV-seronegative volunteers, 7 (78%) elicited a humoral response to Ty after four immunizations with 100 μg of p24-VLP [8]. In the present study, 8 (44%) of 18 HIV-1–infected persons developed Ty antibodies after six immunizations with 100 μg of p24-VLP, and 14 (74%) of 19 participants after 6 immunizations with 500 μg of p24-VLP. The induction of Ty antibodies in most of the participants in the present study illustrates their competence to mount a primary immune response. In HIV-1–infected persons, the persistent high replication rate of HIV and the resultant antigenic presentation of core proteins may lead to a humoral immune response that cannot be improved by immunization with exogenous core antigens.

Boosting of antibodies and the development of antibodies to new epitopes have been induced by HIV envelope subunit vaccine–specific antigens in HIV-infected–persons [12]. The difference in response for recombinant core and envelope antigens might be due to a relative lack of sequence variation in the gag region and the high variation, in and between individuals, in the sequence of the envelope region of HIV-1. In this study, we immunized HIV-1–infected subjects with core antigens that are similar to already circulating antigens. In contrast, subjects immunized with envelope proteins may be exposed to more novel antigens. Different levels of circulating antigens prior to immunization might be another explanation for the difference in responses to envelope and core immunogens; the relatively high level of core antigens is just slightly increased, while the relatively low amount of circulating envelope antigen is increased considerably by immunization.

Vaccination of HIV-infected patients with influenza has been reported to induce an increase in virus load in plasma and peripheral blood mononuclear cells in the first week to 1 month after vaccination [13, 14]. In the present study, we did not see similar changes in the number of HIV-1 RNA copies/mL.

In our study, no evidence of a beneficial therapeutic effect or for accelerated immunologic deterioration due to immunization with p24-VLP was found. No significant changes in laboratory markers for disease progression occurred that could be related to immunization with p24-VLP. Trauger et al. [15] reported increases in p24 antibodies and cell-mediated immune responses that were linked to a reduction in the increase in virus load and CD4 cell decline in asymptomatic HIV-infected persons immunized with envelope-depleted virus. The discrepancy between the results of the latter study and of our present study might reflect differences in antigenicity of the immunogens or might be explained by immune responses to antigens other than p24 or p17.

In conclusion, repeated immunization of HIV-1–infected persons with p24-VLP does not increase p24 antibody levels and has no detectable effect on clinical and laboratory markers for progression of HIV-1 infection.

Acknowledgments

We thank Nel Albrecht-van Lent, Maria Knapen (Municipal Health Service, Amsterdam), John Richens, Fiona Suggett, Diana Aldam (University College London Medical School), Hugo De Vuyst, and Daniel Van den Branden (Institute of Tropical Diseases, Antwerp) for technical assistance; Mark Baillet (Oxford) and Marc Janssen (Amsterdam) for statistical help; Michel Klein (Amsterdam) for critical comments; and Don Jeffries, Jane Hardiman, and Donna Devine for serology (Department of Virology, St. Bartholomews's Hospital Medical School).

References

1. Salk J. Prospects for the control of AIDS by immunizing seropositive individuals. Nature **1987**;327:473–6.
2. Lange JMA, Paul DA, Huisman HG, et al. Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. Br Med J **1986**;293:1459–62.
3. Weber JN, Clapham PR, Weiss RA, et al. Human immunodeficiency virus infection in two cohorts of homosexual men: neutralizing sera an associating of anti-gag antibody with prognosis. Lancet **1987**;1:119–22.
4. Cheingsong-Popov R, Panagiotidi C, Bowcock S, Aronstam A, Wadsworth J, Weber J. Relation between humoral responses to HIV gag and env proteins at seroconversion and clinical outcome of HIV infection. Br Med J **1991**;302:23–6.
5. Hogervorst E, Jurriaans S, de Wolf F, et al. Predictors for non- and slow progression in human immunodeficiency virus (HIV) type 1 infection: low viral RNA copy numbers in serum and maintenance of high HIV-1 p24-specific but not V3-specific antibody levels. J Infect Dis **1995**; 171:811–21.
6. Foster SM, Osborn LM, Cheingsong-Popov R, et al. Decline of anti-p24 antibody precedes antigenaemia as correlate of prognosis in HIV-1 infection. AIDS **1987**;1:235–40.
7. De Wolf F, Goudsmit J, Paul DA, et al. Risk of AIDS related complex and AIDS in homosexual men with persistent HIV antigenaemia. Br Med J **1987**;295:569–72.
8. Martin SJ, Vyakarnam A, Cheingsong-Popov R, et al. Immunization of human HIV-seronegative volunteers with recombinant p17/p24:Ty virus-like particles elicits HIV-1 p24 specific cellular and humoral immune responses. AIDS **1993**;7:1315–23.
9. Taves DR. Minimisation: a new method of assigning patients to treatment and control groups. Clin Pharmacol Ther **1974**;15:443–53.
10. Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S. A rapid and simple PCR assay for quantification of human immunodeficiency virus type 1 RNA in plasma; application to acute retroviral infection. J Clin Microbiol **1994**;32:292–300.

11. Koot M, Vos AH, Keet IPM, et al. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. AIDS 1992;6:49–54.
12. Redfield RR, Birx DL, Ketter N, et al. A phase I evaluation of the safety and immunogenicity of vaccination with recombinant gp160 in patients with early human immunodeficiency virus infection. N Engl J Med 1991;324:1677–84.
13. Ho DD. HIV-1 viraemia and influenza [letter]. Lancet 1992;339:1549.
14. O'Brien WA, Grovit-Ferbas K, Namazi A, et al. Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. Blood 1995;86:1082–9.
15. Trauger RJ, Ferre F, Daigle AE, et al. Effect of immunization with inactivated gp120-depleted human immunodeficiency virus type 1 (HIV-1) immunogen on HIV-1 immunity viral DNA and percentage of CD4 cells. J Infect Dis 1994;169:1256–64.

Maternal Monoclonal Antibody to the V3 Loop Alters Specificity of the Response to a Human Immunodeficiency Virus Vaccine

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The effect of maternally transferred monoclonal antibody (MAb) on the offspring antibody response to $rgp120_{SF2}$ was examined in a murine model. Two MAbs were studied: MAb 83.1, which recognizes a determinant in the V3 loop of gp120 from human immunodeficiency virus-1 (HIV-1) SF2, and MAb 26.2D3, which recognizes a conserved N-terminal region of gp120 from HIV-1_{SF2}. Offspring were immunized at 18–21 days of age with 100 μg of $rgp120_{SF2}$ in complete Freund's adjuvant. Offspring immunized in the presence of preexisting MAb 83.1 but not MAb 26.2D3 demonstrated inhibition of the IgG anti-V3 response. The total IgG anti-$rgp120_{SF2}$ response was not affected by preexisting MAb. Since newborns at risk for HIV may be immunized in the presence of maternal or administered anti-HIV antibody, alternative strategies may be required to circumvent inhibition of the infant's epitope-specific response to HIV immunization by preexisting antibody.

Many strategies are being evaluated to prevent transmission of human immunodeficiency virus (HIV) from an infected mother to her infant [1, 2]. The AIDS Clinical Trials Group (protocol 185) is currently evaluating the effect of HIV immune globulin on maternal transmission of HIV. Ultimately, antibody may be combined with a successful vaccine.

We have developed the murine model for studying effects of preexisting antibody on the murine offspring response to candidate HIV vaccines. In our earlier experiments, we found that maternally transferred polyclonal antibody profoundly inhibited the offspring IgG response to an rgp120 vaccine [3]. In experiments presented here, we demonstrate the effect of maternally transferred monoclonal antibody (MAb) on the subsequent offspring total antibody response and the fine specificity of that response.

Methods

Mice

Specific pathogen–free adult BALB/c mice (Jackson Laboratory, Bar Harbor, ME) were bred for not more than three generations in our laboratory. All immunizations and antibody injections were administered intraperitoneally (ip). MAb was administered to postpartum mice within 24 h after birth of the offspring in a total volume of 0.5–1.0 mL. Antigens were administered in complete Freund's adjuvant (CFA; Life Technologies GIBCO BRL, Gaithersburg, MD) in a total volume of 0.1–0.2 mL. Mice were bled through the retroorbital sinus.

MAb Administered to Postpartum Mice

MAb 83.1 (lot A-2; Repligen, Cambridge, MA) is a BALB/c IgG1 MAb that neutralizes HIV-1_{SF2} [4]. This MAb binds to a determinant defined by amino acids IXIGPGR of the V3 loop of HIV-1 (X can be H, Y, T, R, N, S, or A). MAb 83.1 was prepared by immunization of mice with the Repligen peptide RP142 (YNKRKR.IHI..GPGRAFYTTKNIGC), which is the V3 loop of HIV-

Received 8 January 1996; revised 23 May 1996.
Presented in part: 2nd National Conference, Human Retroviruses and Related Infections, Washington, DC, January 1995 (abstract 521).
All mouse experiments were done according to both institutional and governmental guidelines.
Grant support: Universitywide AIDS Task Force, University of California, NIH training grant (HD-07245), UCLA AIDS Institute seed grant.
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The Journal of Infectious Diseases 1996;174:866–9

0022–1899/96/7404–0030$01.00