CONCISE COMMUNICATION

Human Immunodeficiency Virus Type 1 (HIV-1) gp120–Specific Antibodies in Neonates Receiving an HIV-1 Recombinant gp120 Vaccine

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Infants born to human immunodeficiency virus type 1 (HIV-1)–infected mothers were immunized at birth and at ages 4, 12, and 20 weeks with low-, medium-, or high-dose recombinant gp120 vaccine with MF59 adjuvant (HIV-1_{SF-2}; $n = 52$) or with MF59 alone as a placebo ($n = 9$). An accelerated schedule (birth and ages 2, 8, and 20 weeks) was used for an additional 10 infants receiving the defined optimal dose and for 3 infants receiving placebo. At 24 weeks, anti-gp120 ELISA titers were greater for vaccine-immunized than for placebo-immunized infants on both schedules, and 87% of vaccinees had a vaccine-induced antibody response. At 12 weeks, antibody titers of infants on the accelerated vaccine schedule exceeded those of infants receiving placebo (4949 vs. 551; $P = .01$), and 63% of the vaccinees met the response criteria. Thus, an accelerated schedule of gp120 vaccinations generated an antibody response to HIV-1 envelope distinct from transplacental maternal antibody by age 12 weeks. These results provide support for further studies of vaccine strategies to prevent mother-to-infant HIV-1 transmission.

Although most perinatal human immunodeficiency virus type 1 (HIV-1) infections can be prevented by antiretroviral drug therapy during pregnancy, a substantial fraction of HIV-1–infected pregnant women receive little prenatal care. When drug treatment is delayed until the time of labor, HIV-1 transmission rates are 10%–13% [1, 2]. HIV transmission via breast-feeding remains a problem in developing countries, with transmission rates of 16% during infants' first 2 years of life [3]. Thus, strategies other than drug therapy are needed to prevent mother-to-infant HIV-1 transmission. A vaccine that induces a protective response during the newborn period is likely to be an effective and feasible approach to supplement current interventions.

Immunization at birth prevents certain infectious diseases, such as hepatitis B. In regard to HIV-1 vaccines, it is not known whether the immaturity of the immune systems of newborns

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Informed consent was obtained from the parents and guardians of the children in this study. Guidelines of the US Department of Health and Human Services and of the authors' institutions were followed in the conduct of the clinical research.

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or the presence of maternal HIV-1 antibody might inhibit responses to vaccination during the newborn period. Our study investigated the safety and immunogenicity of a recombinant gp120 vaccine in infants born to infected mothers. This vaccine induces antibody and lymphoproliferative responses in seronegative adults [4–10], and we have shown elsewhere that HIV-1–specific lymphoproliferative responses are induced by the vaccine [11].

Methods

Study design. Pediatric AIDS Clinical Trials Group protocol 230 was a multicenter, phase 1, randomized, placebo-controlled study that tested Chiron Corporation's recombinant gp120 vaccine (strain SF-2) with MF59 adjuvant. Infants born to HIV-1–infected women at ⩾37 weeks of gestation were eligible. In phase 1, patients were randomized into 3 dose-escalating (5, 15, and 50 mg) cohorts receiving vaccine or adjuvant control (placebo). In phase 2, patients were randomized to the 3 dose levels concurrently without placebo. In phases 1 and 2, subjects received immunizations at birth (⩽72 h of age) and at 4, 12, and 20 weeks. The "optimal" vaccine dose was chosen on the basis of maximal cell-mediated immune responses. In phase 3, infants randomized to vaccine or placebo were given the optimal dose (5 mg) on an accelerated schedule (birth and 2, 8, and 20 weeks).

Eighty-seven patients were randomized to receive vaccine or placebo. Antibody data were collected for 80 subjects, 74 of whom were uninfected. These 74 subjects received doses of 5, 15, or 50 mg ($n = 18$, $n = 18$, and $n = 16$, respectively) on the original schedule; 5 mg ($n = 10$) on the accelerated schedule; or placebo on the original ($n = 9$) or accelerated ($n = 3$) schedule.

Of the uninfected subjects with antibody data, 57% were male, 22% were white, 36% were African American, 38% were Hispanic, and 4% were of other ethnicities. The 6 infected infants with repeated positive HIV culture results were similar to the uninfected infants with respect to sex, ethnicity, and median CD4 lymphocyte count (2516 vs. 2202 cells/mm^3), but they had a higher median CD8 lymphocyte count (1357 vs. 806 cells/mm^3; $P = .017$). Standard antiretroviral therapy changed during the trial. In phase 1, zidovudine was administered to 0%, 38%, and 85% of infants receiving the low, medium, and high doses, respectively. In phases 2 and 3, all subjects received zidovudine.

Vaccines. Chiron recombinant (r) gp120 is derived from HIV-1 (strain SF-2) produced in genetically engineered CHO cells. Adjuvant MF59 is an oil-in-water emulsion of 0.5% polysorbate 80, 0.5% sorbitan trioleate, and 0.5% squalene. Both vaccine with adjuvant and adjuvant alone (placebo) were supplied by Chiron and were administered intramuscularly.

Antibody testing. End-point binding antibody titers in plasma were determined by ELISA at Chiron [4]. Serial 2-fold dilutions of plasma, beginning at 1:50, were tested in microtiter plates coated with purified recombinant gp120 and p31 (HIV-1 integrase, SF-2 strain, 2 μg/mL) produced in CHO cells, and yeast, respectively. Bound antibodies were detected by reaction with peroxidase-conjugated goat anti–human IgG and ABTS substrate. The titers (means of duplicates) are reported as the reciprocal of the dilution of serum that gave a half-maximum optical density in the assay. Assays were normalized using the same positive and negative control serum with CHO supernatant and yeast extracts as background controls.

Data analysis. To avoid confounding between responses to vaccine and HIV-1, analyses are restricted to data from uninfected infants, unless specified. The data from vaccinees at all dose levels in phases 1 and 2 were pooled, since the results were consistent. Data for patients receiving placebo on either schedule are combined. Comparisons between patients treated on the original schedule (phases 1 and 2) and the accelerated schedule (phase 3) are potentially confounded, because the patients were enrolled during different time periods.

The traditional definition of a vaccine-induced antibody response as a 4-fold increase in titer from baseline could not be used because of the presence of maternal antibody. Therefore, a vaccine response was defined as a gp120 antibody titer exceeding the titer representing the 99th percentile for the placebo recipients at the particular week. This cut-off titer was 3210 and 1580 for weeks 12 and 24, respectively. Fisher's exact test (2-tailed) was used to compare the fraction of responders for the original versus the accelerated schedule. The Wilcoxon rank sum test was used to compare titers for vaccine versus placebo, original versus accelerated schedule, and baseline characteristics. The Kruskal-Wallis test was used to compare the 3 dose levels. To compare the results of Western blot testing, the Mantel-Haenszel trend test was used with "negative versus indeterminate versus positive" treated as ordered categories.

Results

Antibody responses in uninfected infants. Antibody responses to homologous gp120 were determined at birth and at 12 and 24 weeks of age. The median gp120 antibody titers observed in vaccine recipients were significantly higher than those of placebo recipients after completion of 4 immunizations on either the original or accelerated schedule (table 1). After the 4-dose series, 87% of all vaccine recipients had a vaccine response, as defined in Methods. Subjects on the original schedule received low, medium, or high doses of vaccine. There was no difference in gp120 antibody titer at birth or at 12 or 24 weeks of age in the different dose groups (figure 1*A*). Likewise,

Table 1. Median plasma gp120 antibody titers, as determined by ELISA, for infants who were born to human immunodeficiency virus type 1–infected women and who were receiving gp120 vaccine or placebo.

		Vaccine			
Week	Placebo	Original schedule[a]	P[b]	Accelerated schedule[c]	P[d]
0	4614 (11)	4354 (48)	.62	3412 (8)	.48
12	551 (10)	550 (44)	.78	4949 (8)	.01
24	136 (11)	3848 (46)	<.001	6499 (7)	<.001

NOTE. Data are median titer (no. of subjects tested). Data for low-, medium-, and high-dose vaccine cohorts on the original schedule are combined. Subjects receiving the accelerated schedule received only low-dose vaccine.

[a] Administered at birth and at ages 4, 12, and 20 weeks.

[b] Placebo vs. vaccine on original schedule (Wilcoxon rank sum test).

[c] Administered at birth and at ages 2, 8, and 20 weeks.

[d] Placebo vs. vaccine on accelerated schedule (Wilcoxon rank sum test).

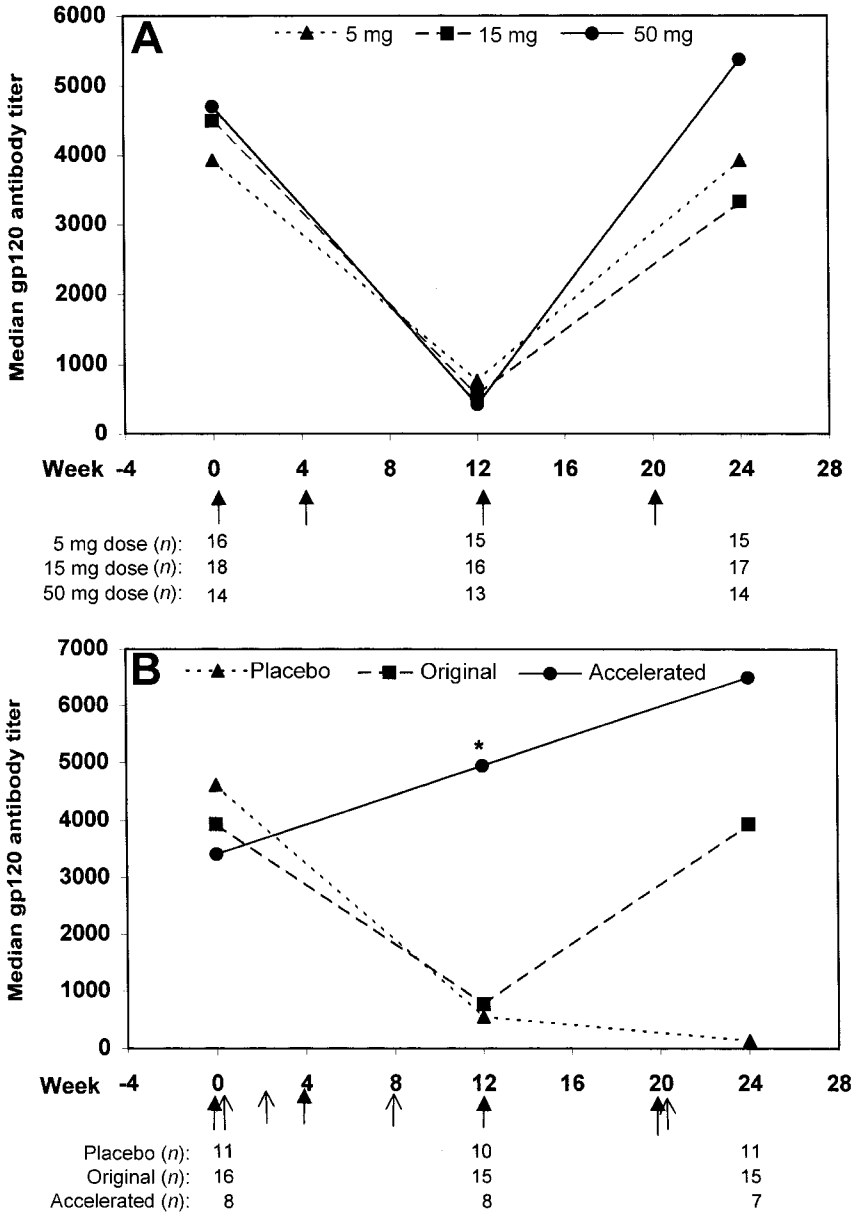

Figure 1. *A,* Median gp120 antibody titers determined by ELISA for neonates receiving low (5 mg), medium (15 mg), or high (50 mg) doses of recombinant gp120 vaccine at birth and at 4, 12, and 20 weeks of age (original schedule; *arrows*). The no. of subjects tested for each dose at each week is specified below the plot. Median values across the doses were not statistically different at any time point ($P > .05$, Kruskal-Wallis test). *B,* Median gp120 antibody titers for neonates receiving 5 mg of vaccine on the original (as defined in panel *A; closed arrows*) or accelerated (birth and 2, 8, and 20 weeks; *open arrows*) schedule and for placebo recipients. *$P < .001$, accelerated vs. original schedule (Wilcoxon rank sum test).

there was no difference in the percentage of responders at 24 weeks (low, 93%; medium, 82%; high, 79%; $P = .60$).

Antibody titers to p31, an HIV-1 protein not present in the vaccine, were determined as an additional control. Titers to p31 declined between birth, 12 weeks, and 24 weeks for both placebo and vaccine recipients (placebo: 6679, 1142, and 589; original schedule: 9112, 822, and 361; accelerated schedule: 5410, 630, and 168).

As expected, almost all subjects with data had positive Western blot results at 0 and 24 weeks (data not shown). Most placebo recipients had negative Western blot results at 52 weeks (4/6 subjects) and 76 weeks (6/6 subjects). By contrast, subjects vaccinated on the original schedule had a greater tendency to maintain a positive or indeterminate Western blot result at 52 weeks (6 negative, 13 indeterminate, and 12 positive; $P = .05$) and 76 weeks (13 negative, 9 indeterminate, and 2 positive;

$P = .058$). At 104 weeks, Western blot results were negative for all 4 placebo recipients and for 19 of the vaccinees for whom data were available (8 other vaccinees had indeterminate results; placebo vs. vaccine; $P = .21$).

Kinetics of the antibody response. Subjects receiving the original regimen had gp120 antibody titers that declined between birth and 12 weeks (figure 1*B*). At 12 weeks, after 2 immunizations, vaccine recipients had gp120 titers that were not different from those of placebo recipients. After 4 immunizations, the gp120 titers in vaccinees increased to levels similar to those detected at birth. By contrast, subjects on the accelerated schedule had no decline in titers between birth and 12 weeks (figure 1*B*). Median antibody titer increased slightly at 24 weeks as a result of additional responders. Among responders at 12 weeks, median titers did not increase between the third and fourth immunization (13,016 vs. 7388). The accelerated schedule yielded titers that were greater than those for the original schedule (limited to 5-mg dose) at 12 weeks (4949 vs. 769; $P \leq .001$), with a marginally significant difference at 24 weeks (6499 vs. 3935; $P = .08$). The percentage of responders was significantly different between the accelerated and the original (limited to the 5-mg dose) schedule at 12 weeks (63% vs. 0%; $P = .002$) but not at 24 weeks (100% vs. 93%; $P = 1.0$).

Of the 6 infected infants receiving vaccine, 5 were on the original schedule. For these infants, median gp120 antibody titers declined at 12 weeks and increased by 24 weeks, with a pattern similar to that of the uninfected vaccinees (median titer, 1796, 340, and 2774 at 0, 12, and 24 weeks, respectively).

Discussion

These results indicate that the candidate rgp120 vaccine was effective in generating binding antibody to gp120. The vaccine also induced lymphoproliferative responses and was well tolerated [11, 12]. The trend toward an increased frequency of positive and indeterminate Western blot results among the vaccine recipients at 52 and 76 weeks suggests a persistent vaccine effect. However, additional testing will be needed to determine the durability of the vaccine-induced antibody responses. Lymphoproliferative responses in some of these vaccinees persisted at age 2 years without additional vaccine boosting [11].

At the initiation of the study, it was not known whether the presence of maternal antibody to HIV-1 would inhibit the generation of HIV-1–specific antibody in response to a vaccine, as has been observed with hepatitis A vaccine [13]. Titers induced by the accelerated schedule when maternal antibody was high were no less than titers induced by the delayed schedule, which suggests that the presence of maternal antibody was not inhibiting. The presence of maternal antibody was not a barrier to evaluating antibody responses to vaccine in infants born to HIV-1–infected mothers. By 12 weeks, maternal antibody had waned sufficiently that antibody generated in response to the vaccine could be detected. Antibody titers to p31, an antigen not present in the vaccine, declined in both vaccinees and placebo recipients, providing further evidence that the envelope antibodies in vaccinees are not the result of persistent maternal antibody.

HIV-1–infected infants who receive combination antiretroviral therapy before age 3 months lose maternal HIV-1 antibody at the same rate as uninfected infants, which suggests that HIV-1–specific antibodies are not synthesized during early infancy [14]. We found that uninfected newborns can generate gp120-specific antibodies by age 3 months. Our data suggest that the lack of early anti–HIV-1 antibody production in infected infants is not due to the presence of maternal antibody or to tolerance induced by in utero exposure but may be specific to the effects of HIV-1 infection on the immune systems of newborns.

Early induction of HIV immune responses is critical to the prevention of perinatal or breast-feeding transmission. We found that an accelerated immunization schedule induced antibody by 12 weeks, provided that 3 immunizations were completed by that time. Seronegative adults receiving this vaccine also require 3 doses to induce high levels of antibody in the absence of priming with live virus vectors [5, 7, 9]. However, adults receiving vaccine on a compressed schedule of monthly immunizations had lower antibody titers than did those on a schedule incorporating a longer rest period [10]. In our study, administering the first 2 doses with only 2 weeks' separation had no inhibitory effect on final titers.

A low vaccine dose was included because the lymphocytes of newborns may respond optimally to lower doses of antigen [15]. Although lymphoproliferative responses were more frequent in the low-dose cohort, antibody titers were not different across doses [11]. In seronegative adults, higher antibody titers were found with 50 rather than 15 mg of vaccine [10]. In our study, a dose 10-fold lower than the 50-mg dose did not reduce the antibody response.

In conclusion, vaccination with recombinant gp120 with MF59 adjuvant induced an antibody response in infants of HIV-1–infected women. An accelerated schedule induced earlier responses with no reduction in the final antibody titers, compared with a regimen in which the second dose was delayed until age 1 month. The vaccine used in this study failed to induce neutralizing antibody to primary HIV isolates in seronegative adults, a characteristic likely to be critical to protective immunity. However, our data demonstrating the capability of newborns to respond to an envelope subunit vaccine should encourage evaluation of other candidate vaccines that may be efficacious in reducing HIV-1 transmission due to peripartum exposure and to breast-feeding in developing nations.

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