MAJOR ARTICLE

Correlation between Immunologic Responses to a Recombinant Glycoprotein 120 Vaccine and Incidence of HIV-1 Infection in a Phase 3 HIV-1 Preventive Vaccine Trial

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(See the article by the rgp120 HIV Vaccine Study Group, on pages 654–65, and the editorial commentary by Graham and Mascola, on pages 647–9.)

Background. An objective of the first efficacy trial of a candidate vaccine containing recombinant human immunodeficiency virus (HIV) type 1 envelope glycoprotein 120 (rgp120) antigens was to assess correlations between antibody responses to rgp120 and the incidence of HIV-1 infection.

Methods. Within the randomized trial (for vaccinees, $n = 3598$; for placebo recipients, $n = 1805$), binding and neutralizing antibody responses to rgp120 were quantitated. A case-cohort design was used to study correlations between antibody levels and HIV-1 incidence.

Results. Peak antibody levels were significantly inversely correlated with HIV-1 incidence. The relative risk (RR) of infection was 0.63 (95% confidence interval, 0.45–0.89) per $\log_{10}$ higher neutralization titer against HIV-1_{MN}, and the RRs of infection for second-, third-, and fourth-quartile responses of antibody blocking of gp120 binding to soluble CD4 versus first-quartile responses (the lowest responses) were 0.35, 0.28, and 0.22, respectively.

Conclusions. Despite inducing a complex, robust immune response, the vaccine was unable to reduce the incidence of HIV-1. Two interpretations of the correlative results are that the levels of antibodies (i) caused both an increased (low responders) and decreased (high responders) risk of HIV-1 acquisition or (ii) represented a correlate of susceptibility to HIV-1 but had no causal effect on susceptibility. Although the data cannot definitively discriminate between these 2 explanations, (ii) appears to be more likely.

The world's first 2 phase 3 HIV-1 vaccine efficacy (VE) trials were completed in 2003 [1, 2]. Both studies tested the efficacy of bivalent vaccines containing recombinant HIV-1 envelope glycoprotein 120 (rgp120) antigens. The first trial (VAX004) was conducted in North America and The Netherlands in 5403 HIV-1–uninfected volunteers, including 5095 non–injection drug using men who have sex with men and 308 women at high risk for heterosexual transmission of HIV-1. The second trial (VAX003) was conducted in 2527 HIV-1–uninfected injection drug users in Bangkok, Thailand [3]. VE for the prevention of HIV-1 infection was estimated as 6% (95% confidence interval [CI], −17% to 24%;

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$P = .59$) in the first trial and as 0% (95% CI, −31% to 24%; $P = .99$) in the second trial, demonstrating lack of efficacy in both populations. In both trials, the rate of HIV-1 infection was approximately constant over time [2].

The vaccines generated antibody responses in nearly 100% of recipients in phase 1 and 2 trials [4–8] and protected chimpanzees from intravenous and mucosal challenge with homologous and heterologous HIV-1 variants [9–11]. The present study undertakes a secondary objective of VAX004: the determination of whether antibody responses to rgp120 correlated with the incidence of HIV-1 infection.

VOLUNTEERS, MATERIALS, AND METHODS

VAX004 trial design. VAX004 was a randomized, double-blinded, placebo-controlled trial. The vaccine consisted of 300 μg each of 2 rgp120 envelope subunits derived from the subtype B isolates MN and GNE8 adsorbed onto 600 μg of alum (AIDSVAX B/B; VaxGen). Volunteers were randomized to receive vaccine or placebo (alum) at a 2:1 ratio. Immunizations were administered by intramuscular injection at months 0, 1, 6, 12, 18, 24, and 30. At each of these visits and at month 36, volunteers were tested for HIV-1 infection by standard HIV-1 ELISA and confirmatory immunoblot. If HIV-1 RNA was undetectable in serum by a highly sensitive and specific nucleic-acid–based amplification test (Procleix HIV-1 Discriminatory Assay) at the date of the last seronegative test, then the date of HIV-1 infection was estimated as the midpoint of the dates between the last negative and first positive ELISA/immunoblot results. Otherwise, the infection date was estimated as the date the earliest sample with detectable HIV-1 RNA was obtained. Greater detail on the study population, counseling procedures, and ethical considerations are provided elsewhere [2].

Anti–HIV-1 antibody assays. Indirect ELISAs of 5 different specificities were used to measure binding antibodies to the vaccine antigen mixture (GNE8/MN rgp120) and to synthetic peptides homologous to the GNE8 V2, MN V2, GNE8 V3, and MN V3 domains of the vaccine antigens (Genentech). Test samples were incubated in duplicate for 2 h in the presence of immobilized antigens at a single fixed dilution that was selected on the basis of the serum responses observed in the AIDSVAX B/B phase 1 and 2 trials, to best resolve the expected range of individual responses. This dilution was 1:50 for the V2 ELISAs and the GNE8 V3 ELISA, 1:500 for the MN V3 ELISA, and 1:5000 for the rgp120 ELISA. Inspection of the serial-dilution profiles of AIDSVAX B/B phase 1 and 2 trial samples by these methods showed them to be parallel, such that, at a fixed dilution, optical density was strongly correlated with end-point titer. Bound antibody was detected on the basis of a 1-h incubation with horseradish peroxidase (HRP)–labeled anti–human IgG (whole molecule) (American Qualex) and colorimetric substrate. Results were normalized and reported as optical densities (rgp120 and MN V3 ELISAs) or as corrected optical densities (V2 and GNE8 V3 ELISAs); for the latter, the optical density from a sample run on a sham-coated plate was subtracted. Two competitive ELISAs were used to measure the antibody blocking of the binding of GNE8 and MN rgp120 to recombinant soluble CD4 (rsCD4; Genentech) [12]. In these competitive ELISAs, biotin-labeled gp120, at an estimated concentration of 125 ng/mL (MN) or 250 ng/mL (GNE8), was immobilized on streptavidin-coated plates. Sample was added at a 1:50 dilution in duplicate and was incubated for 2 h, after which rsCD4 was added (without washing the sample) at a concentration of 500 ng/mL and was incubated for 1 h. Bound CD4 was detected by use of HRP-labeled anti-CD4 monoclonal antibody (Genentech) and colorimetric substrate. Results were normalized and reported as percentage of blocking on the basis of the CD4 binding level in diluent alone. In each of the binding and blocking assays, 2 positive controls, composed of pooled serum samples from AIDSVAX vaccinees, served as the primary system-suitability parameters and were the basis for the normalization of data. A cytopathicity bioassay was used to measure 50% neutralization titers against HIV-1_{MN} [13]. The neutralization assay measured the ability of antiserum to block the cytopathic effect that HIV-1_{MN} has on MT4 cells; MTT dye was used for cell viability readout. Serum samples serially diluted starting at 1:10 were preincubated with virus inoculum before addition to the MT4 cells for a 7-day coculture, and a normalized 50% neutralization titer was reported. For a more complete description of the assays used in the present study, see the Appendix in the electronic edition.

Sequencing of HIV-1 gp120. HIV-1 RNA was isolated from frozen plasma samples obtained at the time of diagnosis of HIV-1 infection, and full-length gp120 genes were amplified by reverse-transcriptase polymerase chain reaction (PCR). PCR products were cloned into a bacterial plasmid, and 3 gp120 clones from each HIV-1–infected volunteer were sequenced at VaxGen.

Schedule of antibody measurements. Serum and plasma samples were obtained from all volunteers at the immunization visits and at the final visit (trough values, at months 0, 1, 6, 12, 18, 24, 30, and 36) and 2 weeks after the immunization visits (peak values, at months 0.5, 1.5, 6.5, 12.5, 18.5, 24.5, and 30.5). For all HIV-1–infected vaccinees, the assays were performed on the peak samples obtained after the last immunization before the estimated date of HIV-1 infection. In addition, for random samples of 5% ($n = 178$) of the vaccinees and 1% ($n = 17$) of the placebo recipients (who were selected before initiation of the trial), the assays were performed on all of the samples obtained at all of the visits. Eleven of the 178 sampled vaccinees became infected with HIV-1 during the trial, and the remaining 167 uninfected vaccinees served as a comparison group for the infected vaccinees. A 5% fraction was chosen because it provided enough uninfected vaccinees for assessment of correlates of HIV-

1 incidence with high power. All of the antibody responses of the placebo recipients were near zero and were not used in the analyses. Antibody responses of samples obtained on or after the estimated date of HIV-1 infection were excluded.

Statistical methods. The Wei-Johnson procedure [14] was used for testing whether an antibody variable differed between groups of vaccinees at 1 or more of the 7 peak time points. Cox proportional hazards models were used to estimate hazard ratios (relative risks [RRs]) of HIV-1 infection for different levels of the most recent preinfection peak antibody response (Borgan et al.'s Estimator I [15] was used). Antibody variables were entered into the model as time-dependent covariates. Except for the neutralization variable, the Cox proportional hazards models that used the actual level (or log level) of response fit poorly, and we therefore focused on models that discretized antibody levels into quartiles (Q1, Q2, Q3, and Q4, with Q1 being the lowest-response quartile). RRs of infection for Q2, Q3, and Q4 versus Q1 were estimated, with and without adjustment for the significant baseline predictors of HIV-1 infection—age (18–25, 26–30, 31–40, 41–50, and >50 years), geographic region (the Midwest, the Northeast, the South, the Southwest, the West Coast, and The Netherlands), and baseline behavioral risk score. The risk score was based on the number of behavioral risk factors for HIV-1 infection that a volunteer self-reported at entry [2].

Because the aforementioned RRs compare groups within the vaccine arm, their interpretation is disconnected from VE. Accordingly, Cox proportional hazards models were used to estimate the RRs of infection, comparing each antibody quartile of vaccinees with the placebo arm. If an rgp120 antibody response is a surrogate of protection (i.e., high antibody levels directly cause a lower susceptibility), then we would expect to observe that (1) the vaccinee infection rate is lower for the higher-response quartiles (Q2–Q4) and (2) the vaccinee infection rate for Q1 is no greater than that for the placebo arm.

On the basis of the assessment of linear correlations among the 8 antibody variables, it appeared that the following 4 variables summarized the essential immunogenicity information: GNE8/MN rgp120, MN neutralization, the average of GNE8 CD4 and MN CD4 blocking (hereafter, "average GNE8/MN CD4 blocking"), and the average of GNE8 V3 and MN V3 binding (hereafter, "average GNE8/MN V3 binding"). Cox proportional hazards models were fit that included these 4 variables simultaneously.

To address the hypothesis that the anti-rgp120 antibodies can recognize only viruses with the same V3 loop tip sequence (GPGRAF) as the GNE8 and MN isolates contained in the vaccine, the RR analyses were repeated for infection with GPGRAF viruses and for infection with non-GPGRAF viruses. In addition, associations between (1) the last peak preinfection antibody levels and (2) the genetic distances between the sequences of the infecting HIV-1 strains and the immunogens were assessed, to determine whether vaccinees with higher antibody levels tended to be infected with relatively divergent HIV-1 strains. Amino acid sequence distances were calculated on the basis of (1) the ~30 discontinuous aa positions representing the neutralizing face core [16]; (2) the positions for (1) plus the ~80 aa positions in the variable loop V2/V3 regions; and (3) the ~33 aa positions in the V3 loop.

All analyses were performed by use of SAS (SAS Institute), R (version 1.9.1), and S-Plus (version 6.2.1; Insightful) software. All P values are 2-sided and are unadjusted for the multiple tests performed, unless stated otherwise. $P < .05$ was considered to be statistically significant.

RESULTS

Antibody responses to rgp120. Of the 241 infected vaccinees, 239 had peak antibody data and were therefore evaluable. Of the 167 randomly sampled uninfected vaccinees, 4 were missing antibody data, and 163 uninfected vaccinees were therefore evaluable. Figure 1 shows individual antibody profiles for a random sample of 10 uninfected vaccinees. For all 8 antibody variables, the mean responses tended to be slightly higher in uninfected vaccinees than in infected vaccinees (figure 2); for GNE8 CD4 blocking and GNE8 V3 binding responses, the differences were significant ($P = .0045$ and $P = .031$, respectively; Wei-Johnson test).

Figure 3 (lower-left panels) shows pairwise scatter plots and Pearson linear correlation estimates of preinfection month 6.5 antibody responses. The GNE8 CD4 and MN CD4 blocking responses and the GNE8 V3 and MN V3 binding responses were strongly correlated, and the GNE8 V2 and MN V2 responses were moderately correlated. The MN neutralization responses were not strongly correlated with any of the other responses. The correlation patterns were similar at the subsequent peak time points (figure 3, upper-right panels). The range of response levels was fairly narrow for some of the assays (figure 3), which limited statistical power for the detection of correlations with infection rate.

Correlation between antibody levels and HIV-1 incidence. Table 1 (left columns) shows the results of the Cox proportional hazards model with the Q1 responses of the vaccinees as the reference group. The incidence of HIV-1 infection was significantly lower in Q2–Q4 responses for GNE8 CD4 blocking, MN CD4 blocking, GNE8 V3 binding, GNE8 V2 binding, and average GNE8/MN CD4 blocking, and there was a nonsignificant trend in this direction for MN neutralization. The CD4 blocking variables best discriminated HIV-1 incidence: the covariate-adjusted RR estimates for the average GNE8/MN CD4 blocking variable were 0.35, 0.28, and 0.22 for Q2–Q4 versus Q1 responses, respectively. On the basis of the multivariable Cox proportional hazards model with average GNE8/MN CD4

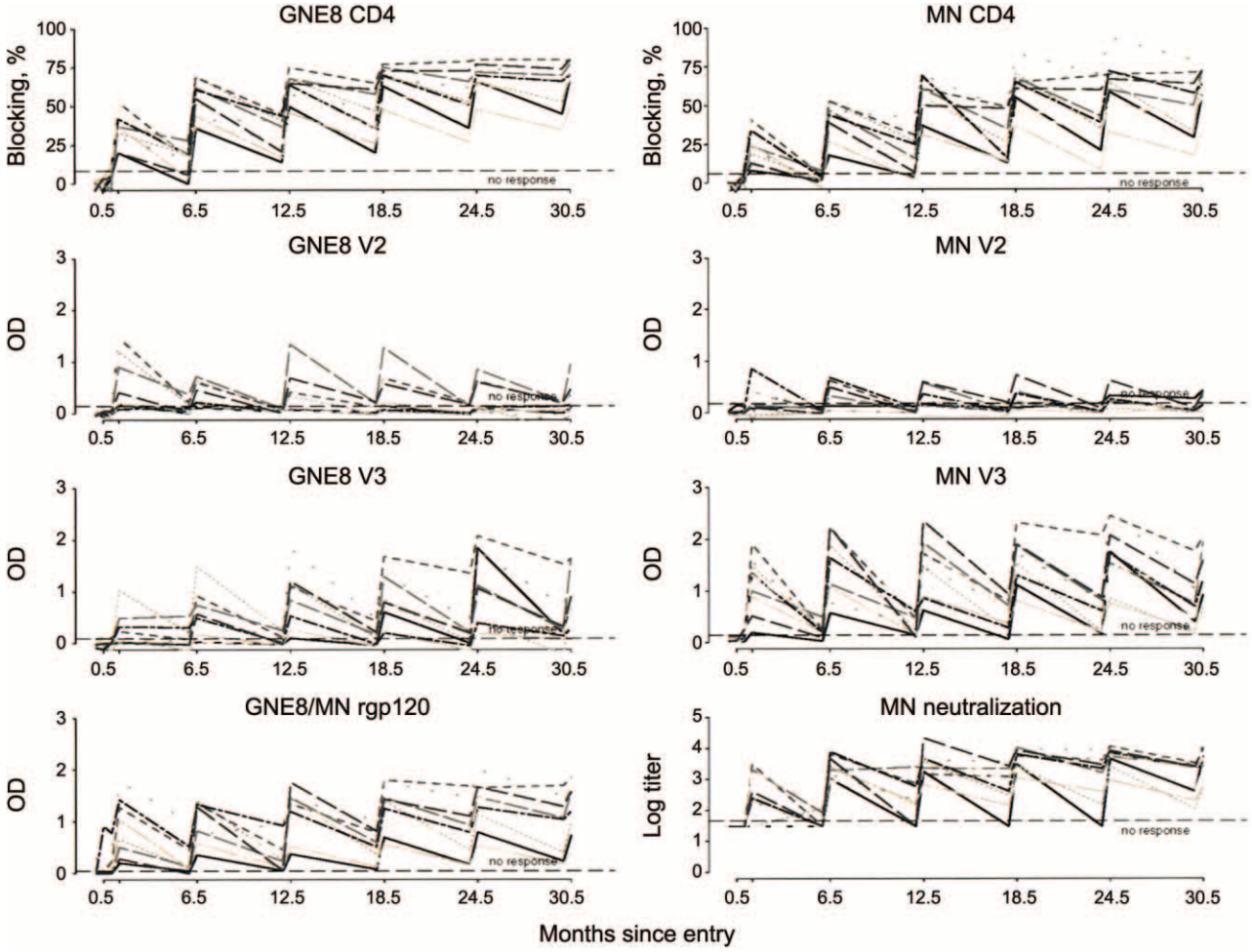

Figure 1. Trough and peak antibody levels, for 10 randomly sampled HIV-1–uninfected vaccinees, for the GNE8 CD4, MN CD4, GNE8 V2, MN V2, GNE8 V3, MN V3, GNE8/MN rgp120, and MN neutralization assays. The lines labeled "no response" indicate negative cutoffs for the 8 assays; 7.7%, 8.6%, 35.9%, 44.1%, 23.0%, 6.7%, 4.8%, and 10.5% of peak responses were negative, respectively.

blocking, average GNE8/MN V3 binding, GNE8/MN rgp120, and MN neutralization quartiles, CD4 blocking was the only significant independent predictor of HIV-1 incidence. Measured as a continuous outcome, the MN neutralization titer was also inversely correlated with HIV-1 incidence, with an RR of 0.63 (95% CI, 0.45–0.89) per $\log_{10}$ higher titer ($P = .0087$) in the univariable model and an RR of 0.71 (95% CI, 0.47–1.06) per $\log_{10}$ higher titer ($P = .091$) in the multivariable model that included the other 3 antibody variables as quartiles.

Table 1 (right columns) shows the results of comparing each response quartile of vaccinees to the placebo arm. For all assays except that for MN V2, the vaccinees with Q1 responses had a greater HIV-1 incidence than did the placebo recipients, although the result was significant only for MN CD4 blocking (RR, 1.78; $P = .026$) and average GNE8/MN CD4 blocking (RR, 1.86; $P = .018$). For the CD4 blocking and V2 assays, vaccinees with Q2–Q4 responses had estimated infection rates that were (nonsignificantly) lower than those in the placebo arm (RRs, 0.73–0.88).

Greater antibody responses in women and nonwhite volunteers. An expanded analysis of immunogenicity was performed in women and nonwhite volunteers by use of the GNE8 CD4 blocking, MN V3 binding, GNE8/MN rgp120, and MN neutralization assays. For all 4 methods, responses were significantly higher in women, with neutralization titers one-half $\log_{10}$ higher on average (data not shown). Responses for the first 3 assays listed above were significantly higher in nonwhite volunteers than in white volunteers, with neutralization titers one-quarter $\log_{10}$ higher on average. For all 8 antibody variables, the response levels were comparable among low-risk (behavioral risk score, 0), medium-risk (behavioral risk score, 1–3), and high-risk (behavioral risk score, >3) vaccinees ($P > .20$, for all).

Correlations between antibody levels and HIV-1 incidence in subgroups. There were nonsignificant trends toward partial

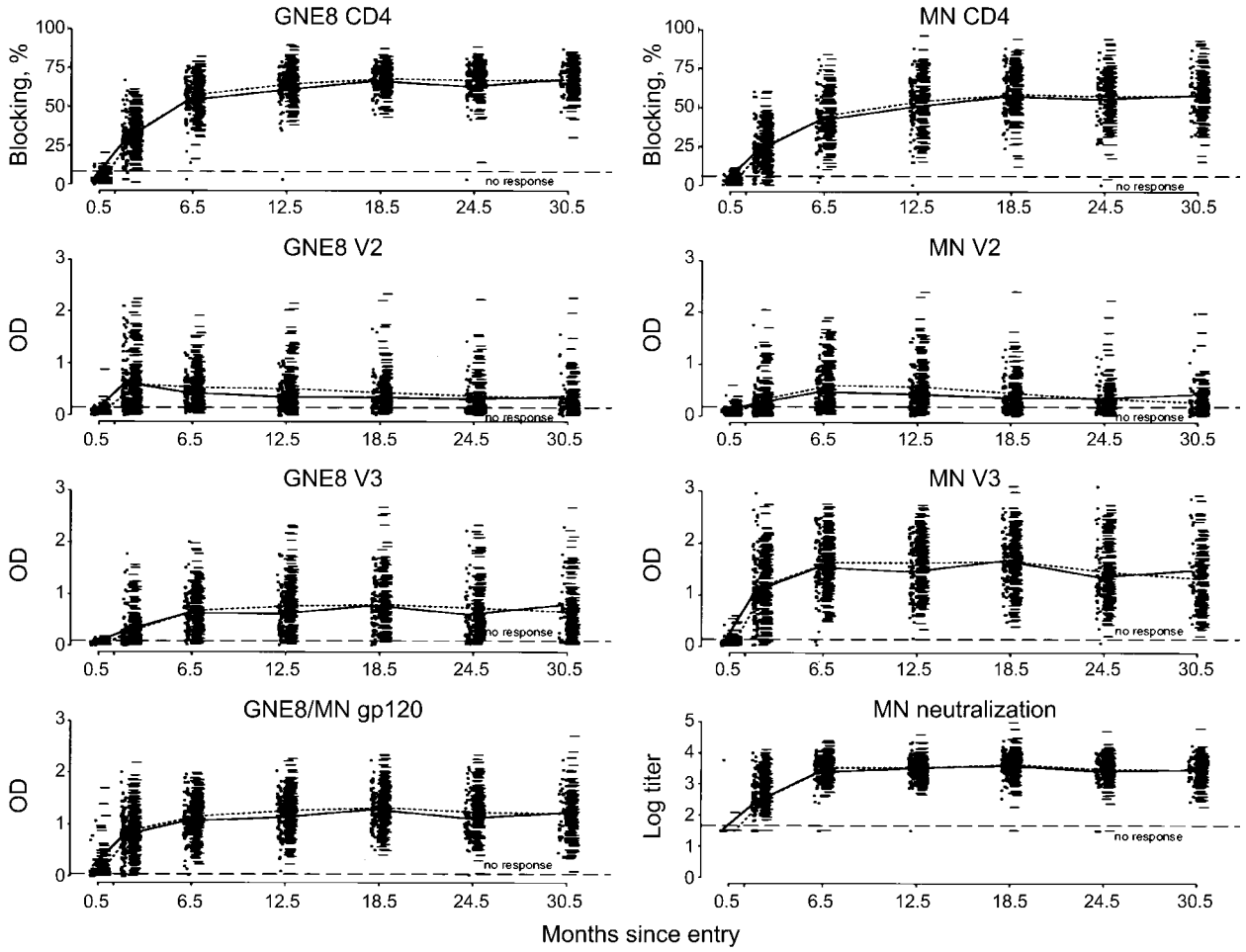

Figure 2. Preinfection peak antibody levels in HIV-1–infected ($n = 239$, denoted by a dot on the left) and HIV-1–uninfected ($n = 163$, denoted by a dash on the right) vaccinees for the 8 immunologic assays listed in figure 1. For infected vaccinees, antibody levels were measured for the last peak sample obtained before the estimated date of HIV-1 infection; for uninfected vaccinees, antibody levels were measured for all 7 peak time points (0.5, 1.5, 6.5, 12.5, 18.5, 24.5, and 30.5). The solid and dotted lines are mean values for the infected and uninfected vaccinees, respectively.

VE in nonwhite and in high-risk volunteers [2]. In exploratory analyses, the Cox proportional hazards model assessments were repeated for race and behavioral risk subgroups. The general pattern of inverse correlations between antibody responses and HIV-1 incidence held in all of the subgroups (table 2 shows the results for white and nonwhite volunteers). The RR estimates for white volunteers were comparable to those for the overall cohort. For nonwhite volunteers, RR estimates for CD4 blocking and V3 binding Q4 responses versus the placebo arm were significantly less than 1 (table 2). However, for all antibody variables, the RR estimates for white volunteers and nonwhite volunteers were not significantly different ($P > .10$, for all). RR estimates were similar among the behavioral low-, medium-, and high-risk subgroups.

We compared the early rgp120 responses of vaccinees among the behavioral risk subgroups, to assess whether the high-risk volunteers may have had natural immunologic priming that was boosted by rgp120. Antibody levels at months 0.5 and 1.5 were similar among the low-, medium-, and high-risk subgroups, which does not support a "prime-boost" hypothesis. However, only 5 high-risk vaccinees had month 0.5 data, and only 20 high-risk vaccinees had month 1.5 data. To fully address the prime-boost hypothesis, future work is planned in which early stored samples from an additional 138 high-risk vaccinees will be assayed.

Correlations between antibody responses and genetic sequences of infecting HIV-1 strains. The results of Cox proportional hazards model analyses were similar when the analyses were restricted to HIV-1 infection with GPGRAF- or non-GPGRAF viruses, which suggests that the correlations between antibody responses and HIV-1 incidence did not depend on the V3 loop tip sequence. For each antibody variable, there

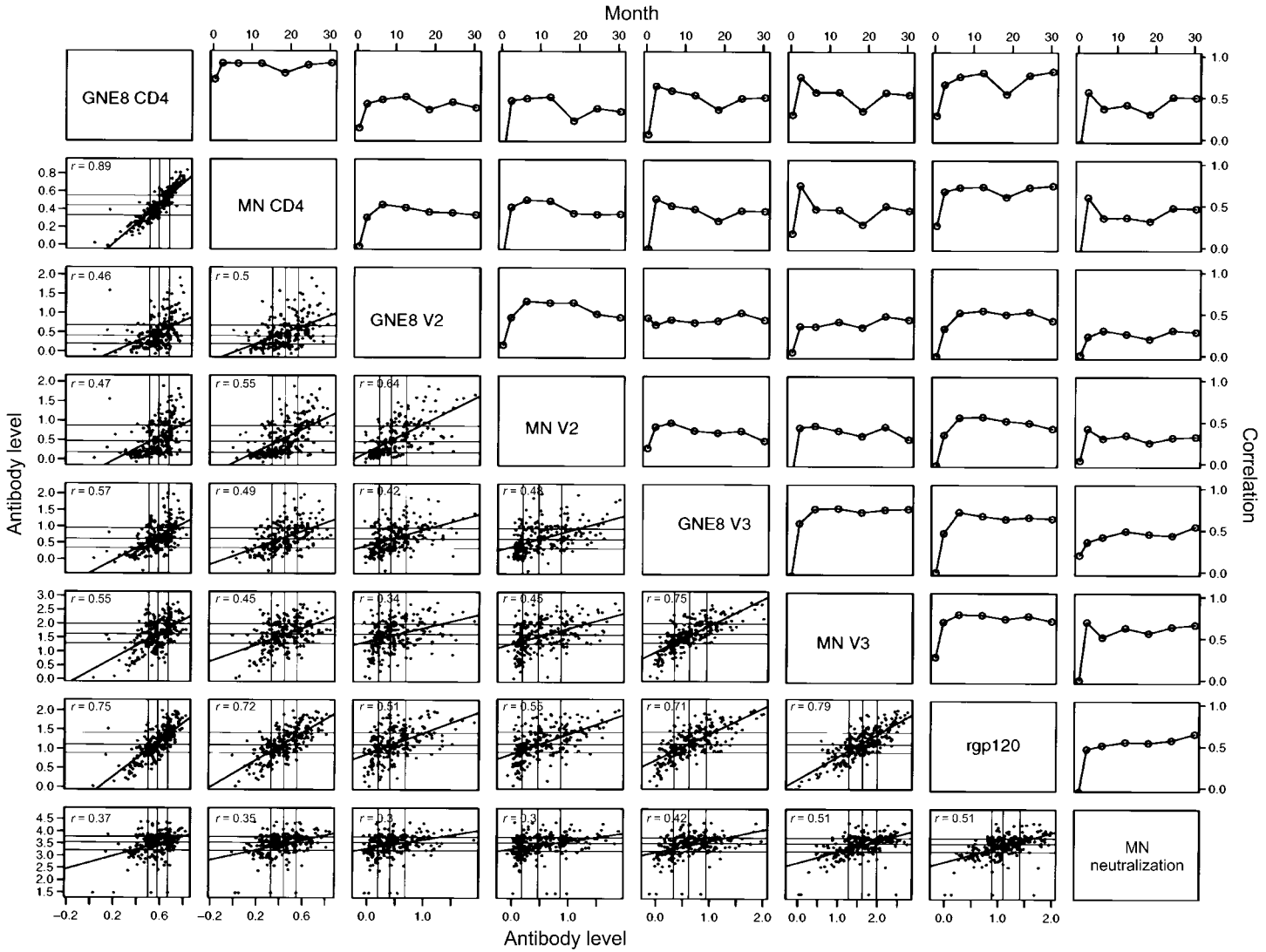

Figure 3. Pairwise scatter plots *(lower-left panels)* of preinfection month 6.5 peak antibody levels for the 8 immunologic assays listed in figure 1. Horizontal and vertical lines denote the 25th, 50th, and 75th percentiles of the preinfection peak antibody levels. Slanted lines are least-squares regression lines. Estimates of the Pearson correlation coefficient, *r,* are given in the upper-left corner of each panel. The upper-right panels show estimates of *r* among pairs of the antibody variables at months 0.5, 1.5, 6.5, 12.5, 18.5, 24.5, and 30.5. For example, the upper-right–most plot shows that GNE8 CD4 blocking and MN neutralization responses had a correlation of 0.0 at month 0.5; a correlation of ~0.5 at month 1.5; a correlation of ~0.4 at months 6.5, 12.5, and 18.5; and a correlation of ~0.5 at months 24.5 and 30.5.

Table 1. For all volunteers, estimated relative risks (RRs) of HIV-1 infection, by quartile of last preinfection peak antibody level, with adjustment for age, region, and behavioral risk score.

	Q1 as reference		Placebo arm as reference	
Immune response, parameter	RR (95% CI)	P^a	RR (95% CI)	P^b
GNE8 CD4 blocking (negative,[c] ≤0.084)		.053 (.024)		
Placebo	…	…	1.00	…
Q1 (−0.35, 0.38)	1.00	…	1.46 (0.87–2.46)	.15
Q2 (0.38, 0.60)	0.48 (0.24–0.98)	.044	1.05 (0.68–1.64)	.83
Q3 (0.60, 0.69)	0.38 (0.17–0.86)	.020	1.00 (0.68–1.48)	.98
Q4 (0.69, 0.89)	0.31 (0.13–0.74)	.008	0.85 (0.56–1.28)	.43
MN CD4 blocking (negative,[c] ≤0.062)		.027 (.019)		
Placebo	…	…	1.00	…
Q1 (−0.09, 0.24)	1.00	…	1.78 (1.07–2.94)	.026
Q2 (0.24, 0.46)	0.46 (0.24–0.87)	.016	0.98 (0.63–1.51)	0.91
Q3 (0.46, 0.61)	0.40 (0.20–0.82)	.012	0.97 (0.65–1.44)	0.87
Q4 (0.61, 0.96)	0.34 (0.16–0.72)	.005	0.87 (0.58–1.30)	0.49
GNE8 V2 (negative,[c] ≤0.148)		.10 (.022)		
Placebo	…	…	1.00	…
Q1 (−0.41, 0.07)	1.00	…	1.49 (0.96–2.31)	.074
Q2 (0.07, 0.23)	0.71 (0.43–1.16)	.17	1.12 (0.76–1.65)	.57
Q3 (0.23, 0.54)	0.66 (0.38–1.14)	.14	1.05 (0.67–1.62)	.84
Q4 (0.54, 2.32)	0.49 (0.28–0.87)	.014	0.73 (0.49–1.11)	.14
MN V2 (negative,[c] ≤0.177)		.072 (.23)		
Placebo	…	…	1.00	…
Q1 (−0.27, −0.07)	1.00	…	0.92 (0.57–1.49)	.74
Q2 (−0.07, 0.21)	1.54 (0.93–2.56)	.10	1.46 (1.00–2.12)	.050
Q3 (0.21, 0.51)	0.94 (0.55–1.63)	.83	0.96 (0.62–1.49)	.85
Q4 (0.51, 2.40)	0.88 (0.48–1.63)	.69	0.88 (0.59–1.30)	.52
GNE8 V3 (negative,[c] ≤0.095)		.035 (.059)		
Placebo	…	…	1.00	…
Q1 (−0.35, 0.09)	1.00	…	1.52 (0.91–2.54)	.11
Q2 (0.09, 0.40)	0.46 (0.25–0.85)	.014	0.84 (0.54–1.33)	.47
Q3 (0.40, 0.87)	0.54 (0.29–1.00)	.051	1.07 (0.74–1.55)	.71
Q4 (0.87, 2.66)	0.41 (0.21–0.80)	.009	0.93 (0.63–1.39)	.73
MN V3 (negative,[c] ≤0.139)		.19 (.46)		
Placebo	…	…	1.00	…
Q1 (0.01, 0.75)	1.00	…	1.25 (0.79–1.97)	.35
Q2 (0.75, 1.34)	0.87 (0.50–1.50)	.62	1.09 (0.72–1.67)	.67
Q3 (1.34, 1.83)	0.59 (0.33–1.07)	.081	0.79 (0.54–1.17)	.24
Q4 (1.83, 3.09)	0.85 (0.46–1.55)	.59	1.12 (0.77–1.63)	.56
MN/GNE8 rgp120 (negative,[c] ≤0.044)		.38 (.14)		
Placebo	…	…	1.00	…
Q1 (−0.01, 0.66)	1.00	…	1.08 (0.62–1.88)	.79
Q2 (0.66, 1.06)	0.99 (0.57–1.70)	.96	1.20 (0.84–1.72)	.32
Q3 (1.06, 1.42)	0.71 (0.39–1.28)	.25	0.90 (0.61–1.32)	.58
Q4 (1.42, 2.69)	0.70 (0.37–1.32)	.27	0.96 (0.65–1.42)	.84
MN neutralization (negative,[c] ≤1.65)		.084 (.096)		
Placebo	…	…	1.00	…
Q1 (1.48, 2.70)	1.00	…	1.60 (0.98–2.63)	.062
Q2 (2.70, 3.38)	0.51 (0.26–0.97)	.040	1.00 (0.67–1.49)	.99
Q3 (3.38, 3.70)	0.42 (0.21–0.83)	.013	0.87 (0.59–1.30)	.51
Q4 (3.70, 4.97)	0.45 (0.22–0.93)	.030	1.02 (0.70–1.47)	.92

(continued)

Table 1. ***(Continued.)***

Immune response, parameter	Q1 as reference		Placebo arm as reference	
	RR (95% CI)	P^a	RR (95% CI)	P^b
Average MN/GNE8 CD4 (negative,[c] ≤0.073)		.026 (.023)		
Placebo	…	…	1.00	…
Q1 (−0.16, 0.31)	1.00	…	1.86 (1.11–3.11)	.018
Q2 (0.31, 0.53)	0.35 (0.16–0.73)	.006	0.99 (0.64–1.55)	.98
Q3 (0.53, 0.66)	0.28 (0.11–0.69)	.006	0.99 (0.67–1.47)	.98
Q4 (0.66, 0.92)	0.22 (0.08–0.61)	.003	0.81 (0.54–1.22)	.32
Average MN/GNE8 V3 (negative,[c] ≤0.17)		.89 (.77)		
Placebo	…	…	1.00	…
Q1 (−0.16, 0.46)	1.00	…	1.35 (0.84–2.14)	.21
Q2 (0.46, 0.90)	0.95 (0.48–1.89)	.88	1.05 (0.67–1.64)	.83
Q3 (0.90, 1.33)	0.84 (0.38–1.82)	.65	0.88 (0.61–1.26)	.48
Q4 (1.33, 2.58)	0.99 (0.41–2.38)	.99	1.02 (0.69–1.52)	.91

NOTE. Quartiles (Q1, Q2, Q3, and Q4, with Q1 being the lowest-response quartile) for the immune-response variables were defined on the basis of all available peak responses. CI, confidence interval.

[a] The first *P* value is for an overall test of any differences in HIV-1 incidence among the 4 quartiles for vaccine recipients; the *P* value in parentheses is for a test for trend for an increasing hazard rate across the quartiles for the immune-response variable. The *P* values in the rows labeled Q2, Q3, and Q4, respectively, are for tests of whether the RRs (hazard ratios) of HIV-1 infection for vaccinees with Q2, Q3, and Q4 responses vs. vaccinees with Q1 responses differed from 1.

[b] The *P* values in the rows labeled Q1, Q2, Q3, and Q4, respectively, are for tests of whether the RRs of HIV-1 infection for vaccinees with Q1, Q2, Q3, and Q4 responses vs. the placebo arm differed from 1. The reference group for the RR results for these comparisons consisted of all 1805 placebo recipients; no antibody data from placebo recipients were used.

[c] Cutoff for negative response, defined as <2 SDs above the mean for serum samples from unvaccinated volunteers.

were no associations between the last preinfection peak antibody level and any of the 3 HIV-1 amino acid distances to GNE8 or MN ($P > .20$, for all; Pearson correlation test).

DISCUSSION

In VAX004, several measurements of peak antibody responses to rgp120 were inversely correlated with HIV-1 incidence. The RR estimates were approximately the same when baseline risk factors were or were not controlled for, suggesting that the associations cannot be explained by imbalances in measured risk factors among vaccinees with high versus low rgp120 responses. The actual level of $\log_{10}$ MN neutralization titer and the average GNE8/MN CD4 blocking response divided into quartiles were the variables most strongly correlated with HIV-1 infection.

In general, across the assays, the vaccinees with low rgp120 antibody responses had a rate of HIV-1 infection higher than that of the placebo recipients, the vaccinees with medium responses had a rate of infection comparable to that of the placebo recipients, and the vaccinees with high responses had a rate of infection lower than that of the placebo recipients. There are 2 possible explanations for this phenomenon: (i) that the responses to rgp120 caused both an increased (in the vaccinees with low antibody responses) and decreased (in the vaccinees with high antibody responses) risk of HIV-1 acquisition or (ii) that the responses to rgp120 marked susceptibility to HIV-1 acquisition but had no causal effect on susceptibility. That is, explanation (i) would imply that the vaccine induced an immune response that enhanced susceptibility to HIV-1 infection in those with low responses, whereas explanation (ii) would imply that the differing antibody responses to rgp120 merely identified the differing capabilities of vaccinees to resist HIV-1 infection. We here consider the relative plausibility of (i) versus (ii).

First, note that, for a variable to be identified as a surrogate of protection within a trial, it is necessary that the vaccine have substantial efficacy [17], which was not observed. Correlation analyses that used the placebo arm as the reference population illustrated this point—for example, RR estimates for Q1, Q2, Q3, and Q4 average GNE8/MN CD4 blocking responses of vaccinees versus placebo recipients were 1.86, 0.99, 0.99, and 0.81, respectively. If this variable were a surrogate of protection, then the RR estimate for Q4 would be substantially and significantly less than 1. For none of the antibody variables did the RR estimate for Q4 versus the placebo arm differ significantly from 1, thereby supporting (ii), not (i).

The analysis of VE and rgp120 levels in relation to the genetic sequences of the infecting HIV-1 strains also supports (ii) over

Table 2. For white and nonwhite volunteers, estimated relative risks (RRs) of HIV-1 infection vs. the placebo arm, by quartile of last preinfection peak antibody level, with adjustment for age, region, and behavioral risk score.

	White volunteers		Nonwhite volunteers	
Immune response, parameter	RR (95% CI)	P^a	RR (95% CI)	P^a
GNE8 CD4 blocking (negative,[b] ⩽0.084)		.14 (.066)		.27 (.17)
Placebo	1.00	…	1.00	…
Q1 (−0.35, 0.38)	1.70 (0.95–3.01)	.072	1.23 (0.36–4.19)	.74
Q2 (0.38, 0.60)	1.30 (0.80–2.12)	.29	0.54 (0.21–1.40)	.21
Q3 (0.60, 0.69)	1.10 (0.72–1.67)	.67	0.73 (0.28–1.93)	.53
Q4 (0.69, 0.89)	1.05 (0.67–1.67)	.82	0.30 (0.10–0.87)	.027
MN CD4 blocking (negative,[b] ⩽0.062)		.041 (.069)		.31 (.19)
Placebo	1.00	…	1.00	…
Q1 (−0.09, 0.24)	2.20 (1.29–3.74)	.004	1.37 (0.35–5.45)	.65
Q2 (0.24, 0.46)	1.17 (0.72–1.89)	.52	0.56 (0.21–1.49)	.25
Q3 (0.46, 0.61)	1.04 (0.67–1.61)	.86	0.75 (0.31–1.86)	.54
Q4 (0.61, 0.96)	1.10 (0.70–1.71)	.68	0.28 (0.10–0.79)	.016
GNE8 V2 (negative,[b] ⩽0.148)		.029 (.0064)		.27 (.40)
Placebo	1.00	…	1.00	…
Q1 (−0.41, 0.07)	2.00 (1.22–3.29)	.006	0.26 (0.06–1.22)	.088
Q2 (0.07, 0.23)	1.36 (0.89–2.09)	.16	0.45 (0.17–1.21)	.11
Q3 (0.23, 0.54)	1.12 (0.69–1.81)	.64	0.97 (0.40–2.39)	.95
Q4 (0.54, 2.32)	0.84 (0.53–1.33)	.46	0.51 (0.20–1.26)	.14
MN V2 (negative,[b] ⩽0.177)		.062 (.27)		.50 (.21)
Placebo	1.00	…	1.00	…
Q1 (−0.27, −0.07)	1.04 (0.62–1.72)	.89	0.78 (0.23–2.60)	.68
Q2 (−0.07, 0.21)	1.77 (1.18–2.67)	.006	0.82 (0.33–2.05)	.67
Q3 (0.21, 0.51)	1.15 (0.71–1.86)	.58	0.46 (0.17–1.21)	.12
Q4 (0.51, 2.40)	1.04 (0.67–1.60)	.86	0.44 (0.17–1.17)	.099
GNE8 V3 (negative,[b] ⩽0.095)		.099 (.070)		.17 (.53)
Placebo	1.00	…	1.00	…
Q1 (−0.35, 0.09)	1.74 (1.00–3.03)	.049	1.23 (0.43–3.54)	.70
Q2 (0.09, 0.40)	1.07 (0.65–1.75)	.80	0.30 (0.10–0.89)	.030
Q3 (0.40, 0.87)	1.15 (0.77–1.74)	.49	0.93 (0.39–2.24)	.88
Q4 (0.87, 2.66)	1.15 (0.74–1.76)	.54	0.38 (0.14–1.08)	.070
MN V3 (negative,[b] ⩽0.139)		.082 (.83)		.53 (.26)
Placebo	1.00	…	1.00	…
Q1 (0.01, 0.75)	1.47 (0.89–2.44)	.14	0.82 (0.25–2.72)	.75
Q2 (0.75, 1.34)	1.27 (0.80–2.01)	.30	0.64 (0.26–1.59)	.34
Q3 (1.34, 1.83)	0.87 (0.57–1.32)	.51	0.72 (0.30–1.75)	.47
Q4 (1.83, 3.09)	1.49 (0.98–2.27)	.063	0.34 (0.13–0.88)	.027
MN/GNE8 rgp120 (negative,[b] ⩽0.044)		.73 (.28)		.19 (.31)
Placebo	1.00	…	1.00	…
Q1 (−0.01, 0.66)	1.29 (0.70–2.36)	.41	0.80 (0.24–2.70)	.72
Q2 (0.66, 1.06)	1.32 (0.88–1.97)	.18	0.92 (0.39–2.17)	.85
Q3 (1.06, 1.42)	1.15 (0.75–1.75)	.53	0.27 (0.09–0.81)	.020
Q4 (1.42, 2.69)	1.13 (0.74–1.73)	.57	0.51 (0.19–1.37)	.18
MN neutralization (negative,[b] ⩽1.65)		.048 (.016)		.61 (.48)
Placebo	1.00	…	1.00	…
Q1 (1.48, 2.70)	2.11 (1.23–3.63)	.007	0.67 (0.18–2.45)	.54
Q2 (2.70, 3.38)	1.10 (0.71–1.70)	.67	0.80 (0.32–1.99)	.63
Q3 (3.38, 3.70)	1.01 (0.66–1.57)	.95	0.44 (0.17–1.12)	.085
Q4 (3.70, 4.97)	1.26 (0.84–1.89)	.26	0.46 (0.17–1.23)	.12

(continued)

Table 2. ***(Continued.)***

Immune response, parameter	White volunteers		Nonwhite volunteers	
	RR (95% CI)	P^a	RR (95% CI)	P^a
Average MN/GNE8 CD4 (negative,[b] ≤0.073)		.061 (.063)		.16 (.073)
Placebo	1.00	…	1.00	…
Q1 (−0.16, 0.31)	2.17 (1.24–3.77)	.006	1.82 (0.51–6.50)	.36
Q2 (0.31, 0.53)	1.21 (0.74–1.98)	.45	0.61 (0.23–1.59)	.31
Q3 (0.53, 0.66)	1.09 (0.71–1.67)	.69	0.70 (0.28–1.79)	.46
Q4 (0.66, 0.92)	1.02 (0.65–1.60)	.92	0.28 (0.10–0.80)	.017
Average MN/GNE8 V3 (negative,[b] ≤0.17)		.59 (.96)		.43 (.29)
Placebo	1.00	…	1.00	…
Q1 (−0.16, 0.46)	1.58 (0.94–2.66)	.085	0.97 (0.32–3.00)	.96
Q2 (0.46, 0.90)	1.23 (0.76–1.98)	.40	0.58 (0.22–1.56)	.28
Q3 (0.90, 1.33)	0.95 (0.63–1.43)	.81	0.71 (0.31–1.64)	.42
Q4 (1.33, 2.58)	1.33 (0.87–2.04)	.19	0.32 (0.11–0.93)	.036

NOTE. Quartiles (Q1, Q2, Q3, and Q4, with Q1 being the lowest-response quartile) for the immune-response variables were defined on the basis of all available peak responses. CI, confidence interval.

[a] The first *P* value is for an overall test of any differences in HIV-1 incidence among the 4 quartiles for vaccine recipients; the *P* value in parentheses is for a test for trend for an increasing hazard rate across the quartiles for the immune-response variable. The *P* values in the rows labeled Q1, Q2, Q3, and Q4, respectively, are for tests of whether the RRs of HIV-1 infection for vaccinees with Q1, Q2, Q3, or Q4 responses vs. the placebo arm differed from 1. The reference group for the RRs for white volunteers consists of all 1495 white placebo recipients, and the reference group for the RRs for nonwhite volunteers consists of all 310 nonwhite placebo recipients; no antibody data from placebo recipients were used.

[b] Cutoff for negative response, defined as <2 SDs above the mean for serum samples from unvaccinated volunteers.

(i). There were no associations between the last peak preinfection antibody levels and the genetic distances between the sequences of the infecting HIV-1 strains and the immunogens, and match or mismatch of the infecting HIV-1 strains to the GPGRAF V3 loop tip sequence did not affect the degree of correlation between antibody levels and HIV-1 incidence. Furthermore, VE did not significantly vary with any of the amino acid distances or with match or mismatch to GPGRAF. Nearly all of the HIV-1 strains sampled at the time of diagnosis of infection were substantially different from both GNE8 and MN with respect to gp120 amino acid sequence, suggesting that the measured responses to GNE8 and MN are unlikely to be reliable surrogates for rgp120 responses to circulating HIV-1 isolates. The extensive antigenic heterogeneity of the infecting HIV-1 strains and the inability of rgp120 to induce antibodies that neutralize primary HIV-1 strains likely played an important role in the failure of rgp120 to confer protection, pointing to the need for vaccine constructs that induce broader and more complex immune responses.

The low biological plausibility that low rgp120 responses would enhance the risk of HIV-1 infection further supports (ii). Enhancement is a theoretical concern for the rgp120 vaccine [18–20], and vaccine-induced partial immunity has been observed to increase the severity of several infectious diseases caused by infection with enveloped viruses [21–27]. However, in phase 1 and 2 trials of rgp120, there was no in vitro evidence of antibody-dependent enhancement [5]; also, other than one possible exception [28], we are not aware of any clinical or animal studies that have clearly demonstrated an antibody-mediated increased susceptibility to acquisition of infection. Furthermore, in both VAX004 and VAX003, viral loads, $CD4^+$ lymphocyte counts, and times to initiation of antiretroviral therapy were similar in HIV-1–infected vaccinees and placebo recipients and did not correlate with antibody response, suggesting that rgp120 did not enhance disease.

The data from white volunteers could also support (ii). Lack of efficacy in white volunteers was established with high confidence (VE, −6% [95% CI, −35% to 16%]), yet, even in this subgroup, the rgp120 responses were inversely correlated with HIV-1 incidence.

The exploratory analyses of the subgroups with a nonsignificant trend toward VE (i.e., the behavioral high-risk subgroup and the nonwhite subgroup) also seemed more supportive of (ii) than (i). Response levels were comparable across behavioral risk levels, so there was no evidence that high behavioral risk vaccinees had greater immune responses that could have conferred some protection. Levels for 4 antibody variables were modestly and significantly higher in nonwhite volunteers than in white volunteers; however, for all antibody variables, the RRs did not significantly differ between nonwhite volunteers and white volunteers. Explanation (ii) for nonwhite volunteers is supported by the fact that rgp120 responses were

only modestly higher in nonwhite volunteers than in white volunteers and by the fact that (ii) is strongly supported in white volunteers.

Although (ii) appears more likely than (i), definitive discrimination between these explanations would require either data from vaccinees on a variable (or variables) that is correlated with the rgp120 responses, is unaffected by rgp120, and does not interfere with the immune responses induced by rgp120 or data from placebo recipients on a variable that predicts how they would have responded to the vaccine. For example, if all trial volunteers had been immunized with another recombinant-protein vaccine to which they were naive (e.g., an experimental recombinant anthrax vaccine), then the relationship between the anthrax and rgp120 responses in vaccinees could be used to impute to each placebo recipient an rgp120 response that he or she would have had if vaccinated. This would allow direct testing of (i) versus (ii) on the basis of data. Indeed, a lesson learned from VAX004 is that, in future efficacy trials, it may be important to collect additional data to aid the analyses of immune responses. One variable that might help is a measure of the magnitude of clonality within the T lymphocyte repertoire [29].

In summary, certain antibody responses to the rgp120 vaccine do appear to have predictive value for susceptibility to HIV-1 infection, although they likely do not have any direct effect on susceptibility to HIV-1 infection. Some intrinsic host genetic mechanisms that confer some protection against HIV-1 infection have been described [30, 31]. In addition, resistance to HIV-1 infection has been described in "highly exposed, seronegative" sex workers [32–36]. The differing HIV-1 acquisition rates we observed may not be related to either of these mechanisms. Because the development of an HIV-1 vaccine has been hampered by the lack of clear correlates of immunity [37], it would seem important to further investigate the phenomenon we describe, for it might lead to knowledge of what is required to produce an effective vaccine. In accordance with this, the rgp120 HIV Vaccine Study Group is conducting additional analyses using stored VAX004 samples, including analyses of host genetics, of additional rgp120 responses measured immediately after the first vaccination (which could indicate immune priming), of coinfection with GB virus C [38], of T lymphocyte responses, and of the ability of serum from vaccinees to neutralize a large panel of diverse primary isolates.

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