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Recombinant gp120 Vaccine-Induced Antibodies Inhibit Clinical Strains of HIV-1 in the Presence of Fc Receptor-Bearing Effector Cells and Correlate Inversely with HIV Infection Rate

Donald N. Forthal, Peter B. Gilbert, Gary Landucci, and Tran Phan

Nonneutralizing Abs may play a role in protecting animals and humans from lentiviral infections. We explored the Ab-dependent, cell-mediated virus inhibition (ADCVI) Ab response to recombinant gp120 (rgp120) vaccination in sera from 530 participants in the Vax 004 trial. Serum ADCVI activity was measured against a clinical R5 strain of HIV-1 using peripheral blood mononuclear effector cells from healthy donors. The level of vaccine-induced ADCVI activity correlated inversely with the rate of acquiring HIV infection following vaccination, such that for every 10% increase in ADCVI activity, there was a 6.3% decrease in the hazard rate of infection ($p = 0.019$). Some vaccinated individuals also mounted an ADCVI response against two other clinical R5 strains of HIV-1. However, ADCVI activity correlated poorly with neutralizing or CD4-gp120-blocking Ab activity measured against laboratory strains. Finally, the degree to which the ADCVI Ab response predicted the rate of infection was influenced by polymorphisms at the FcγRIIa and FcγRIIIa gene loci. These data indicate that rgp120 vaccination can elicit Abs with antiviral activity against clinical strains of HIV-1. However, such activity requires the presence of FcR-bearing effector cells. Our results provide further evidence that ADCVI may play a role in preventing HIV infection. The Journal of Immunology, 2007, 178: 6596–6603.

Antibodies protect nonhuman primates from lentivirus infection and may be essential components of vaccine-induced immunity against HIV in humans (1–7). However, the biological activity of Ab responsible for protection in vivo is unknown. Although neutralizing Ab activity often correlates with protection (3, 5, 8, 9), nonneutralizing Abs also prevent or modulate SIV and SHIV infections, and the fact that Ab neutralizes cell-free virus in vitro does not necessarily implicate neutralization as the critical Ab function in vivo.

Ab-dependent cell-mediated virus inhibition (ADCVI) occurs when Ab forms a bridge between an infected target cell and an FcR-bearing effector cell (10, 11). This interaction results in reduced virus yield due to target cell death from Ab-dependent cellular cytotoxicity (ADCC), triggering of soluble antiviral factors, or FcγR-mediated phagocytosis of immune complexes (11, 12). ADCVI Ab is measurable early during acute HIV infection and correlates inversely with plasma viral load (11). ADCVI Abs are also elicited by infection or immunization with SIV (13). Finally, nonneutralizing Ab that prevents SIVmac251 infection after oral challenge of newborn rhesus macaques has potent ADCVI activity against the challenge strain (13). Thus, ADCVI may be an immunological correlate of protection against lentivirus infections.

Two phase III trials showed that bivalent, recombinant gp120 (rgp120) vaccines did not prevent HIV infection (14, 15). The first of these trials, Vax 004, was conducted primarily in the United States among men who have sex with men (14). The vaccine used consisted of rgp120 from two laboratory-passaged strains of HIV-1, HIV-1MN, and HIV-1GNE8. Although no vaccine efficacy was demonstrated, a separate analysis revealed an inverse correlation between vaccine-induced Ab activity and HIV infection rate (16). The authors suggested that vaccine-induced Ab was a marker of some other, presumably innate, immune response that ultimately reduced the rate of infection. The possibility that vaccine-induced Ab itself was directly linked with protection was also considered (16). These analyses used Abs measured in binding or functional assays against laboratory-passaged strains of HIV-1, including those used in the vaccine. If Abs were causally associated with prevention following vaccination, it is likely that such Abs would have antiviral activity against clinical HIV-1 strains. In this study, we determined whether vaccination with rgp120 during the Vax 004 trial could elicit ADCVI Ab against clinical strains of HIV-1. We also determined whether ADCVI Abs correlated with HIV-1MN-neutralizing Abs or with Abs that block binding of CD4 to HIV-1GNE8 gp120. The potential role of ADCVI Abs in preventing infection was determined by correlating ADCVI activity with the rate of infection among vaccinated individuals. Finally, because ADCVI is an FcR-dependent immune function, we explored the relationship between polymorphisms in the FcγRIIa and FcγRIIIa genes, ADCVI Ab activity, and infection rate.

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3 Abbreviations used in this paper: ADCVI, Ab-dependent cell-mediated virus inhibition; ADCC, Ab-dependent cellular cytotoxicity; CI, confidence interval.

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Materials and Methods

Study subjects

This research was approved by the University of California, Irvine, Institutional Review Board. Serum samples from 530 participants who received vaccine in the Vax 004 trial were used to measure ADCVI activity. Vaccinations were given at months 0, 1, 6, 12, 18, 24, and 30 (14). Samples were obtained from all participants who became infected during the trial and from whom serum was available (n = 231). Uninfected subjects were chosen on the basis of a 5% random sampling of all uninfected subjects and supplemented by an additional 65 subjects considered to have high-risk behaviors (total number of uninfected subjects = 299). All but two samples were from men, 385 (73%) participants considered themselves white, and 145 (27%) considered themselves African American, Hispanic, Asian, or other. For uninfected vaccinees, ADCVI activity was measured in samples collected 2 wk following the fourth vaccination, which occurred at month 12 after enrollment in Vax 004; Ab levels are considered to have peaked 2 wk after each vaccination (16). For infected vaccinees, ADCVI activity was measured on samples collected 2 wk after the last immunization before the estimated date of infection; ADCVI activity was not assayed on any sample obtained after infection. Eighty-two (15%) of these samples were drawn 2 wk after the first, second, or third immunization, 341 (64.3%) after the fourth immunization, and 175 (32.7%) after the fifth, sixth, or seventh immunizations. For each subject, a specimen from a single time point was used. To compare ADCVI activity in infected and uninfected subjects, analyses were performed using the specimens obtained after the fourth, fifth, sixth, or seventh immunizations. This was justified by the finding that Ab measured 2 wk after vaccination reached maximum levels after the third immunization and remained at about the same level with successive vaccinations (16). Using specimens obtained after earlier vaccinations would have biased the analyses by creating lower Ab levels for infected vaccinees due to earlier sampling than for uninfected vaccinees. Month 12.5 specimens from 30 randomly chosen uninfected placebo recipients were also assayed for ADCVI activity.

ADCVI assay (11)

Target cells for the ADCVI assay were prepared by infecting polybrene-treated CEM.NKR-CCR5 cells (National Institutes of Health AIDS Research and Reference Program) with a clinical strain of HIV-1 (HIV-192US657) at a multiplicity of infection of 0.05. After 48 h, target cells were washed to remove cell-free virus. Effector cells (PBMCs from healthy donors) were added to target cells at an E:T ratio of 10:1. Test sera were added to target and effector cells to achieve a final dilution of 1/100. Seven days later, supernatant fluid was collected, and p24 was measured by ELISA (Zeptometrix). The percent inhibition due to ADCVI was calculated relative to pooled HIV-negative sera from CEM.NKR-CCR5 cells. Individual sera of v2, v5, or v6 were assayed in two separate assays with different donors, and the mean percent inhibition was calculated in general. Values from the two assays were in close agreement with each other. Specimens whose SD was >20% (16% of the samples) were repeated a third time, and the mean of all three assays was used.

Adsorption with gp120

Sera from 10 vaccinated subjects were adsorbed with HIV-1MN gp120 (Vaxgen) and subsequently assayed for ADCVI activity against HIV-1MN gp120. Individual wells of a 24-well flat-bottom plate (Costar) were coated with either 250 μg of gp120 or 250 μg of BSA by overnight incubation at 4°C. Plates were washed three times with PBS and blocked for 2 h at room temperature with blocking buffer (5% nonfat dried milk and 5% sucrose in PBS). Plates were washed three times with PBS, and 250 μl of serum (1/10 dilution) was added, and plates were incubated at 37°C on a rotator platform for 1 h. Serum was collected, and the process repeated before vaccination and calculation of ADCVI activity at a final concentration of 1/100. To determine the extent to which anti-gp120 Abs were removed by adsorption, gp120-expressing 8E5 cells were incubated with either gp120-adsorbed sera or sera adsorbed with BSA. Additional controls consisted of the 8E5 cells incubated with HIV-negative or unadsorbed positive serum. After washing, FITC-conjugated goat anti-human IgG was added, and the cells were washed further. Cytometry was then used to measure the percentage of FITC-positive cells.

HIV-1MN-neutralizing and CD4-gp120-blocking Ab

HIV-1MN-neutralizing Ab titers were measured by the ability of Ab to protect MT4 cells from HIV-1MN-induced cytoxicity, as described previ-ously (17). A competitive ELISA was used to measure the ability of Ab to block the binding of HIV-1MN rgp120 to soluble CD4 (16); results are reported as the percent binding in the presence of serum (1/50) relative to binding in the presence of diluent. Both the neutralizing and the blocking Ab activities were measured as part of a previously published study, and the results were made available to us for correlation with ADCVI activity (16).

FcyRIIa and IIIa genotyping

DNA was extracted from PBMCs using the Qiagen DNeasy Tissue kit (Qiagen) according to the manufacturer’s instructions. Genotyping was performed using methods based on those of Dall’Ozzo et al. (18). Sample DNA was used to amplify the region of the FcyRIIa gene spanning the single nucleotide polymorphism at position 494. A common forward primer (5′-CAATTTGCTGCTATGGGC-3′) was used in combination with the histidine (H) (5′-GCGGGCAGGGCGGCGGGGCGGCCA-3′) or arginine (R) allele primer (5′-ATCCAGAAATTTCTCCCG-3′) in separate 25-μl reactions. The H allele primer included a 26-base GC tail. DNA and primers were added to Applied Biosystems SYBR Green PCR Mastermix (Applied Biosystems), and amplification was performed with a Rotogene 3000 thermocycler using the following parameters: 95°C for 10 min, followed by 40 cycles at 95°C (60 s), 57.5°C (30 s), and 72°C (60 s). Once amplification was complete, melt curves were obtained with +0.5°C stepwise increments (72–95°C) of 10 s each. Positive controls for all genotypes were included in each assay; the genotypes of control samples were verified by sequencing the FcyRIIa gene in the region of the polymorphism.

FcyRIIa genotypes were determined using a similar strategy. A common forward primer (5′-TCCAAAGCCACATCTCAAAGTC-3′) was used with either V-specific (5′-AGACACATTATTACCTCATT-3′) or F-specific (5′-GCGGGCACAGGCCGGCGGGCGGGCGGGTAGTGTACAGCTCTGCTAGCATACACATTTTACTCCCATTA-3′) reverse primers. The following thermocycler parameters were used: 95°C for 10 min, followed by 50 cycles at 95°C (20 s), 59°C (60 s), and 72°C (45 s).

Statistical analyses

Distributions of Ab levels among vaccinated groups were compared using Kruskal-Wallis tests. Spearman rank tests were used to evaluate correlations between different Ab assays. The relative hazards of HIV infection by Ab activity (entered into the model as a continuous variable or in quartiles) were estimated using Cox proportional hazards models. Models were adjusted for race (white or nonwhite), geographic region, and behavioral risk score (14). The case-cohort sampling design, wherein Ab activity was measured in all available infected vaccinees and a random sample of uninfected vaccinees, was accommodated using Estimator II (19); this accounts for enriched sampling of high-risk participants. Wald tests within the Cox model were used to evaluate interactions between variables.

Results

Immunoization with rgp120 elicits ADCVI Ab against a clinical HIV-1 strain

We determined ADCVI Ab responses 2 wk after immunization with HIV-192US657, a clinical R5 strain, in the sera of 530 Vax 004 subjects immunized with rgp120. A wide spectrum of ADCVI Ab activity was elicited by the vaccine (Fig. 1), with 27% of sera obtained on or after month 12.5 having ≥90 and 12% of sera demonstrating ≥95% inhibition at a dilution of 1/100. Inhibition was dependent on the presence of the PMBC effector cells, since in their absence, there was little or no serum antiviral activity (Fig. 2a). Month 12.5 sera from 30 randomly selected placebo recipients were also assayed, using pooled HIV-negative serum as a reference; essentially, no ADCVI activity was measurable (maximum inhibition = 5%, average inhibition = 1.5%).

To rule out a “prozone” effect, where Ab activity is inhibited at higher concentrations of serum, nine samples with high, low, or medium ADCVI activity were assayed at serial 2-fold dilutions between 1/50 and 1/1600. No sera exhibited a prozone effect (Fig. 2b), which indicates that the percent inhibition at 1/100 is representative of the ADCVI activity in each serum. Even sera with high activity at 1/100 lost activity at a dilution of 1/400 (Fig. 2b).
ADCVI Ab activity correlates poorly with HIV-1MN-neutralizing absorption with gp120 (data not shown). was a only with control (BSA-coated) wells (Fig. 3). By cytometry, there activity by an average of 87%, as compared with sera incubated with gp120 (data not shown).

**ADCVI Ab activity correlates poorly with HIV-1MN-neutralizing or CD4-gp120-blocking Abs**

As reported previously, vaccine-induced HIV-1MN-neutralizing or CD4-gp120-blocking Ab levels were inversely associated with infection rate in the Vax 004 trial (16). We compared these Ab results with ADCVI Ab activity, using samples from the 253 participants for whom all three Ab measurements were available. For subjects with neutralizing or blocking Ab results available after multiple immunizations, the earliest value after the fourth immunization was used. ADCVI activity did not correlate with either of the other two Ab measurements (Spearman rank correlation \( r = 0.02, p = 0.81 \) for ADCVI vs HIV-1MN-neutralizing and \( r = 0.10, p = 0.13 \) for ADCVI vs CD4-gp120-blocking Ab; Fig. 4, a and b). However, HIV-1MN-neutralizing titers and CD4-gp120-blocking Ab activity correlated with each other (\( r = 0.36, p < 0.001; \) Fig. 4c).

**The rate of HIV infection after vaccination correlates inversely with ADCVI Ab activity**

We next determined the relationship between infection rate and the ADCVI Ab response to rgp120. ADCVI activity was measured against HIV-1MN gp120 using sera from all uninfected subjects obtained 2 wk after the fourth vaccination (i.e., month 12.5). Subjects who subsequently became infected had ADCVI activity measured in sera obtained just before their date of infection; some of these subjects were infected before month 12.5. To avoid comparing sera obtained after three or fewer vaccinations among the infected subjects with those obtained after four or more vaccinations in uninfected subjects, we analyzed data from sera obtained only after four or more vaccinations \( (n = 299 \) uninfected and 149 infected subjects). Those who remained uninfected had higher ADCVI Ab activity than subjects who became infected \( (p = 0.04, \) Kruskal-Wallis test; Fig. 5). We also analyzed data from sera obtained only after month 12.5 for both infected \( (n = 42) \) and uninfected \( (n = 299) \) subjects. Again, uninfected subjects had higher ADCVI activity than infected subjects \( (p = 0.004; \) data not shown).

We next estimated hazard ratios for acquiring HIV infection after vaccination for different levels of ADCVI Ab activity. In this and in subsequent analyses, ADCVI results from all serum specimens obtained after month 12.5 were used. Using a Cox proportional hazard model controlling for behavioral risk score, race, and geographic region, ADCVI Ab, as a continuous variable, was inversely associated with infection rate (hazard ratio = 0.937 for each 10% increase in ADCVI activity; 95% confidence interval \( (CI) = 0.89–0.99; p = 0.019; \) Table I). Thus, for every 10% increase in ADCVI activity, there was a 6.3% decrease in rate of infection. Subjects in the fourth quartile had a significantly lower...
rate compared with those in the first quartile (hazard ratio = 0.54; 95% CI 0.29–0.97; \( p = 0.035 \)). These results indicate that ADCVI Ab responses are inversely associated with the rate of HIV infection following vaccination with rgp120.

**Immunization with rgp120 elicits an ADCVI Ab response against other clinical strains in some subjects**

The inverse association between ADCVI activity and HIV infection rate raised the possibility that rgp120 elicits ADCVI Ab capable of inhibiting a variety of circulating strains of HIV-1. We randomly selected sera from 70 infected and 70 uninfected vaccinees and measured ADCVI activity against two additional clinical subtype B R5 strains of HIV-1, HIV-192US712, and HIV-191US005. The amino acid homology between the gp120s of the three strains ranged from 84 to 87% (www.pubmed.gov). Again, a wide range of ADCVI Ab responses were observed against the two additional strains (Fig. 6). ADCVI activity against HIV-192US712 was generally lower than against HIV-192US657 or HIV-191US005. There was a significant correlation between ADCVI activity against HIV-191US005 and HIV-192US712 (\( r = 0.38; p < 0.001 \)), whereas there was very poor correlation between the activity against HIV-192US657 and either HIV-191US005 (\( r = 0.047; p = 0.58 \)) or HIV-192US712 (\( r = -0.12; p = 0.15 \)). Although the correlation between ADCVI activity against HIV-192US657 and the other clinical strains was poor, 55% of the samples reactive against HIV-192US657 (≥50% inhibition) were also reactive against HIV-191US005; 24% of samples reactive with HIV-192US657 were also reactive against HIV-192US712. Thus, for some subjects, vaccination with rgp120 resulted in ADCVI activity that cross-reacted with diverse strains of HIV-1. As with HIV-192US657, there was no significant correlation between ADCVI activity against either HIV-191US005 or HIV-192US712 and anti-HIV-1MN- neutralizing or CD4-gp120-blocking Abs (data not shown). In this small, randomly selected subset of the total specimens, there

**Figure 4.** Serum ADCVI Ab activity against a clinical R5 strain of HIV-1 (HIV-192US657) does not correlate with neutralizing Ab titers against the laboratory strain HIV-1MN (a) or with gp120-CD4-blocking Ab activity against the laboratory strain HIV-1MN (b). The neutralizing and blocking Ab activities against the two laboratory strains do correlate with each other (0.36, \( p < 0.001 \)) (c); the regression line is shown.

**Figure 5.** Subjects who remained uninfected during the VaxGen 004 trial had higher serum ADCVI activity against a clinical R5 strain of HIV-1 than subjects who became infected (\( p = 0.04 \), Kruskal-Wallis test). Sera were assayed at a dilution of 1/100. For uninfected vaccinees, ADCVI activity was measured in samples collected 2 wk following the fourth vaccination, which occurred at month 12 after enrollment in Vax 004; Ab levels are considered to have peaked 2 wk after each vaccination. For infected vaccinees, ADCVI activity was measured on samples collected 2 wk after the last immunization before the estimated date of infection. Horizontal lines on box plots delineate quartiles.

Table 1. Relationship between ADCVI Ab activity and rate of HIV infection among vaccinees in the Vax 004 trial

<table>
<thead>
<tr>
<th>ADCVI</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>0.94(^b)</td>
<td>0.89–0.99</td>
<td>0.019</td>
</tr>
<tr>
<td>Fourth vs first quartile</td>
<td>0.54</td>
<td>0.29–0.97</td>
<td>0.035</td>
</tr>
</tbody>
</table>

\(^a\) ADCVI values were entered into the Cox proportional hazard model as either a continuous variable or as quartilized data. The model also included behavior risk scores, ethnicity, and geographic region.

\(^b\) A 6% decrease in infection rate for every 10% increase in ADCVI activity.
were no significant associations between Ab activity against any of the virus strains and infection rate.

FcγRIIa and FcγRIIIa gene polymorphisms influence the ADCVI Ab response to rgp120 and modulate the relationship between ADCVI Ab and infection rate

ADCVI depends on the binding of Ab to IgG Fc receptors (FcγRs) on NK cells, monocytes, or macrophages, and such binding is affected by polymorphisms in the genes encoding two of these receptors, FcγRIIa and FcγRIIIa (20–24). FcγRIIa is encoded by a gene having two alleles resulting in H or R at aa 131 (21, 25). The H allele is required for binding to IgG2 and increases binding to IgG3 (21, 22). FcγRIIIa is also encoded by a gene having two alleles, resulting in valine (V) or phenylalanine (F) at aa 148 (26). The V isoform binds better to IgG1 or IgG3 than does the F isoform (26–28). We explored relationships between FcγRIIa/IIIa genotypes, ADCVI Ab responses, and HIV infection rates. We first determined whether FcγR genotypes affected the ADCVI Ab response. This analysis did not take infection status into consideration, and data from all subjects with Ab measured on or after month 12.5 and with genotyping results for FcγRIIa (n = 281) or FcγRIIIa (n = 282) were used. There was a statistically significant association between FcγRIIa genotype and ADCVI activity, such that individuals with the HH genotype had the highest activity, those with the RH genotype had intermediate activity, and those with the RR genotype had the lowest activity (p = 0.03, Kruskal-Wallis test; Fig. 7). The HH and RR genotypes differed significantly from each other (p = 0.01, Kruskal-Wallis test), as did the HH and RH genotypes (p = 0.03); there was no difference between the RH and RR genotypes (p = 0.42). There was also a difference in ADCVI Ab levels by FcγRIIIa genotype (p = 0.03), with significantly higher activity among the VVs compared with the FVs (p = 0.007) and compared with the FFs (p = 0.03). Thus, for both FcγRIIa and IIIa, subjects with genotypes resulting in the highest affinity receptors had the highest ADCVI Ab responses to the vaccine.

Based on interaction tests, we also found that the FcγRIIa gene modified the effect of ADCVI Ab activity on the rate of HIV infection for ADCVI activity entered as a continuous variable (p = 0.01) or as quartiles (p = 0.009; Table II). Using ADCVI as a continuous variable, the hazard ratio among subjects with one or two R alleles was 0.89 per 10% increment of ADCVI activity.
Table II. Modulation of the relationship between ADCVI activity and HIV infection rate by FcγRIIa and IIIa genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ADCVI</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR or RH</td>
<td>Continuous 0.89</td>
<td>0.83–0.96</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>Continuous 0.92</td>
<td>0.78–1.07</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>FcγRIIla</td>
<td>Fourth vs first quartile 0.55</td>
<td>0.10–3.12</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>FV or VV</td>
<td>Continuous 0.94</td>
<td>0.86–1.04</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fourth vs first quartile 0.43</td>
<td>0.16–1.16</td>
<td>0.088</td>
<td></td>
</tr>
</tbody>
</table>

a ADCVI values were entered into the Cox proportional hazard model as a continuous or quantized variable. The model also included behavior risk scores, ethnicity, and geographic region.

b An 11% decrease in infection rate for every 10% increase in ADCVI activity.

c A 14% decrease in infection for every 10% increase in ADCVI activity.

(95% CI = 0.83–0.96; p = 0.002). For subjects who were homozygous for the H allele, the hazard ratio was 0.92 (95% CI 0.78–1.07; p = 0.25). When comparing the highest with the lowest Ab quartile, the hazard ratios were 0.30 (95% CI 0.12–0.71; p = 0.005) for subjects with any R allele and 0.55 (95% CI 0.10–3.12; p = 0.49) for subjects homozygous for the H allele. There were too few subgroups homozygous for the R allele to evaluate that group separately. Thus, the presence of an R allele resulted in the most benefit from high ADCVI activity, whereas homozygosity for the H allele reduced or abrogated that benefit.

There was also an interaction between FcγRIIIa and ABCI activity in predicting infection rate for ADCVI activity entered as a continuous variable (p = 0.02) or as quartiles (p = 0.06; Table II). Among subjects homozygous for the F allele, there was a significant inverse relationship between ADCVI activity (as a continuous variable) and infection rate (hazard ratio = 0.86 per 10% increment in ADCVI activity; 95% CI 0.78–0.95; p = 0.003). In contrast, the inverse association between ADCVI activity and infection rate was less apparent and not significant among heterozygous FVs (hazard ratio = 0.94; 95% CI 0.84–1.05; p = 0.25) or when FVs and VVs were combined (hazard ratio = 0.94; 95% CI 0.86–1.04; p = 0.24). There were too few samples to analyze separately. Similarly, when comparing the highest quartile ADCVI Ab responses with the lowest quartile, there was a significant inverse association between HIV rate among Ff subjects (hazard ratio = 0.32; 95% CI 0.26–0.38; p = 0.018), a strong but nonsignificant trend among FV subjects (hazard ratio = 0.33; 95% CI 0.10–1.10; p = 0.06) and a nonsignificant trend among those with either the FV or VV genotypes (hazard ratio = 0.43; 95% CI 0.16–1.16; p = 0.088). Thus, the homozygous F genotype resulted in the most benefit from high ADCVI activity, and the presence of a V allele may have reduced that benefit.

Because of linkage disequilibrium between the FcγRIIa and IIIa genes, we used two interaction terms in a Cox model to determine whether each gene independently influenced the relationship between ADCVI activity and infection (29). In that analysis, with ADCVI as a continuous variable, FcγRIIa independently influenced the relationship (p = 0.002) whereas FcγRIIIa did not (p = 0.16).

**Discussion**

We explored the relationship between the ADCVI Ab response to rgp120 vaccine and the rate of HIV infection. We found that the level of vaccine-induced ADCVI activity correlated inversely with the rate of acquiring HIV infection following vaccination. In addition, vaccinated individuals mounted a variable ADCVI response against clinical R5 strains of HIV-1, and this response did not correlate with neutralizing or CD4-gp120-blocking assays measured against laboratory strains of virus. Finally, the degree to which the ADCVI Ab response predicted the rate of infection was influenced by polymorphisms at the FcγRIIa and FcγRIIIa gene loci.

Vaccination with rgp120 elicited an ADCVI Ab response against clinical R5 strains of HIV-1 in the majority of vaccinated subjects. The absence of an appreciable ADCVI response in sera from placebo recipients indicates that the vaccine itself elicited the ADCVI Ab. Furthermore, adsorption with rgp120 removed almost all of the ADCVI activity. Thus, ADCVI Ab was directed largely or exclusively against gp120. These results indicate that vaccination with rgp120 from laboratory strains of HIV-1 can elicit an Ab response that inhibits clinical strains of HIV-1. Notably, the same vaccine gives negligible neutralizing Ab activity against a panel of clinical HIV-1 isolates (D. Montefiori, unpublished observation). This discordance might be explained by the fact that the panel used for neutralizing assays did not include the strains used in our ADCVI assays. However, discordance between ADCVI or ADCC and neutralizing activities has been observed previously and may be due to differences in antigenic targets or in the subclass distribution of the two functional Ab responses (13, 30, 31). Alternatively, the Ag-Ab affinity required for ADCVI might be less than that required for neutralization because ADCVI Ab need not block virus-receptor interactions. Finally, ADCVI, unlike neutralization, requires an interaction between Fcs and FcγRs on effector cells and is measured against infected cells rather than against cell-free virions. It is likely that the cross-linking of Fcs, with consequent target cell lysis and chemokine triggering, underlies the antiviral activity of these vaccine-induced Abs (11).

The ADCVI Ab response correlated inversely with HIV infection rate. As was proposed for the vaccine-induced Ab responses reported by Gilbert et al. (16), it is possible that ADCVI activity is a correlate for some other unmeasured immune response that is independent of vaccination and causally related to protection. However, it is possible that vaccine-induced ADCVI Ab itself provided some degree of protection. We previously showed that ADCVI Abs arise early and correlate inversely with the viremia level during acute HIV infection (11). Nonneutralizing anti-SIV-Ab that prevents infection of neonatal macaques after oral SIVmac251 challenge has potent ADCVI activity (13); in the absence of other Ab functions, it is likely that ADCVI accounted for this protection. Recently, a vaccine-induced reduction in acute SIV viremia was shown to correlate with the ADCC Ab response to the vaccine (32). Death of infected target cells by ADCC is one of the mechanisms by which ADCVI inhibits HIV-1 (11). These studies, together with our current results, support the possibility that ADCVI is an important protective immunological function against lentiviral infection.

The most compelling argument in support of a direct role for ADCVI in preventing infection comes from our demonstration that polymorphisms in FcγR genes modified the relationship between ADCVI Ab and infection rate. FcγRs bind IgG or immune complexs and trigger phagocytosis, the release of chemokines and cytokines, ADCC, and ADCVI (11, 33, 34). FcγRIla is found on several cell types involved in HIV infection, including monocytes, macrophages, and dendritic cells. The H allele of FcγRIla is required for binding to IgG2 and also increases binding to IgG3 (21, 22). FcγRIIa is the predominant FcγR on NK cells and is also found on some monocytes, macrophages, and dendritic cells (23, 35–37). The V isoform of FcγRIIa binds better to IgG1 or IgG3 than does the F isoform (26–28). In our study, subjects with lower affinity receptors (RR or HR in the case of FcγRIIa and FF in the case of FcγRIIIa) were more likely to benefit from higher serum
ADCVI activity than those with higher affinity receptors. The fact that FcγR polymorphisms modulate the effect of Ab on infection rate supports a direct role for Ab in preventing infection because the natural ligand for FcγRs is IgG. However, it should be noted that FcγRIIIa binds to C-reactive proteins, and other ligands may exist as well (38, 39). In addition, it is possible that linkage between the FcγRs and other genes could underlie some of our observed associations. However, there are no known associations between polymorphisms in FcγR genes and other genes. In contrast, there is a linkage between the FcγRIIIa and FcγRIIIa genes, such that the higher affinity alleles of both genes are more commonly found together (29); this linkage may account for the fact that in a Cox model, including genotypes from both FcγRIIIa and FcγRIIIa, only the FcγRIIIa genotype significantly influenced the relationship between ADCVI and infection rate.

It is unclear why vaccinees with lower affinity receptors would benefit most from higher ADCVI Ab. Because we did not use autologous combinations of subjects’ sera and effector cells, we can’t conclude that genotype exerted its effect on infection risk by increasing or decreasing overall ADCVI activity. In fact, it is likely that genotype modulated this relationship through a mechanism other than ADCVI itself. Otherwise, we might expect the high-affinity genotypes (VVVs and HHs) to be associated with a lower risk of infection at any given level of serum ADCVI activity because the higher affinity genotypes mediate better ADCC activity (40, 41). In contrast, high-affinity genotypes may enhance the risk of infection through an Ab-dependent mechanism, as suggested by the finding that infants homozygous for FcγRIIIa H have at least a 2.2-fold increased risk of perinatal HIV infection compared with infants having one or no H allele (42). We propose that our results reflect opposing Ab functions: ADCVI on the one hand and virus enhancement or blocking of beneficial functions in contrast. Thus, in the absence of high-affinity receptors, enhancement or blocking is less likely to occur, and antagonical functions such as ADCVI predominate. However, in the presence of high-affinity receptors, enhancement or blocking may negate any beneficial Ab function, even though effector cells with such receptors are best able to carry out ADCVI. Our data also suggest a role for vaccine-induced IgG2 or IgG3 in enhancement or blocking because binding of those subclasses are most impacted by the FcγRIIIa polymorphism (21, 22). Further in vitro and in vivo studies will be required to test these possibilities. It should be emphasized that the Vax 004 trial overall showed no efficacy. However, it is difficult to explain why, despite the lack of correlation between ADCVI activity and blocking or neutralizing Abs, ADCVI was also inversely associated with infection rate. One possibility is that the ADCVI and the neutralizing/blocking Ab responses are both causally and independently associated with preventing infection. However, despite the ability of serum from vaccinees to neutralize HIV-1MN, they generally do not neutralize primary strains of HIV-1 (D. Montefiori, unpublished observation). Thus, it seems unlikely that HIV-1MN neutralization itself is directly related to infection rate. Alternatively, one Ab function may be causally associated, whereas the others may be a correlate for some other protective immune response. Finally, all of the Ab measurements may be such correlates; although our data cannot rule in or out this possibility, as discussed above, we show some evidence consistent with a direct role for ADCVI in preventing infection.

We also found that vaccination with rgp120 elicits ADCVI Abs against three different clinical R5 strains. However, correlations between the ADCVI response against HIV-1R5b and against the other strains was poor. This suggests that rgp120-induced ADCVI Ab is directed against variable epitopes that differ on the three strains. Given the inverse relationship between anti-HIV-1MN activity and infection rate, it is possible that HIV-1R5b is more representative of circulating HIV-1 strains than are the other two strains. Only a small proportion of the total sera were assayed against the other strains; with a larger sample size, intercorrelations between all three strains or inverse correlations between infection rate and all three strains might have become evident.

In summary, we found that the level of vaccine-induced ADCVI activity against a clinical R5 strain of HIV-1 correlated inversely with the rate of HIV infection following vaccination with rgp120. The degree to which the ADCVI Ab response predicted the rate of infection was influenced by polymorphisms in FcγR gene loci. These results are consistent with a role for ADCVI in preventing HIV infection. In vitro, mechanistic studies and additional analyses of large vaccine trials will be necessary to further sort out the implications of Fc-FcγR interactions in preventing HIV-1 infection.

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Disclosures

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References


