Phase I studies of volunteers not infected with human immunodeficiency virus type 1 (HIV-1) have shown that immunization with envelope subunit vaccine products elicits antibodies that neutralize laboratory-adapted (prototype) HIV-1 strains in vitro. Prototype strains are adapted to grow in continuous (neoplastic) cell lines and are more susceptible to neutralization than are primary isolates cultured in human peripheral blood mononuclear cells. In this study, 50 sera from nine phase I vaccine trials and 16 from HIV-1-infected persons were evaluated for neutralizing antibody activity against 3 laboratory-adapted and 5 primary HIV-1 isolates. Of 50 sera, 49 neutralized at least 1 of the prototype strains; however, none displayed neutralizing activity against primary isolates of HIV-1. Serum from most HIV-1-infected persons neutralized both laboratory-adapted and primary HIV-1 isolates. These data demonstrate a qualitative, or large quantitative, difference in the neutralizing antibody response induced by envelope subunit vaccination and natural HIV-1 infection.

In several viral diseases, neutralizing antibodies are thought to be an important component of a protective immune response [1–8]. Initial preclinical human immunodeficiency virus type 1 (HIV-1) vaccine studies demonstrated that antiserum elicited by immunization with HIV-1 envelope glycoproteins gp120 or gp160 or by peptides derived from the third variable region (V3) of gp120 could neutralize HIV-1 in vitro [9–11]. In addition, chimpanzees vaccinated with recombinant envelope glycoprotein (rgp) vaccine products or passively immunized with a neutralizing monoclonal antibody to the V3 region of gp120 were protected from infection when challenged with HIV-1 [12–14]. The current focus by vaccine manufacturers on envelope subunit vaccines is due, in large part, to the successful chimpanzee experiments described above, neutralization assays used laboratory-adapted strains of HIV-1 that have been cultured and propagated in human neoplastic cell lines. In contrast, primary HIV-1 isolates are cultured only in uninfected human peripheral blood mononuclear cells (PBMC). HIV-1 enters cells via binding to the CD4 receptor, and a soluble form of CD4 (sCD4) can neutralize HIV-1 in vitro. In 1990, it was demonstrated that neutralization of HIV-1 primary isolates required ~100- to 1000-fold higher concentrations of sCD4 than did neutralization of laboratory-adapted (prototype) strains [16, 17]. Subsequent studies have shown that, compared with primary HIV-1 isolates, laboratory-adapted strains are markedly more sensitive to neutralization by polyclonal sera from HIV-1–infected persons and by neutralizing monoclonal antibodies [18–21]. The underlying mechanism for the relative neutralization sensitivity of prototype strains is not well established [22–25], although a similar phenomenon has been described for other retroviruses [26, 27].

Numerous phase I studies using HIV-1–seronegative human volunteers have demonstrated that immunization with rgp160, rgp120, and V3 peptide products can elicit neutralizing antibod-
ies to laboratory-adapted strains of HIV-1 [28–35]. In these studies, primary viruses were not tested. Because of the established disparity in neutralization susceptibility, selected sera from nine phase I trials of HIV-1 envelope subunit candidate vaccine products were evaluated for neutralizing antibody activity against a panel of 3 laboratory-adapted and 5 primary isolates of HIV-1.

Materials and Methods

Subjects, vaccine preparations, and study protocols. Study volunteers were healthy adults between 18 and 60 years of age who were at low risk for HIV infection and were negative for HIV-1 by ELISA. Volunteers were enrolled into one of nine multicenter safety and immunogenicity trials of candidate HIV-1 envelope-derived subunit vaccines conducted by the National Institute of Allergy and Infectious Diseases–sponsored AIDS Vaccine Evaluation Group (AVEG); the trials have been described in detail elsewhere [29, 31, 33, 34, 36–40]. Table 1 summarizes each candidate vaccine, including information on manufacturers, virus strain, dose, adjuvant, and immunization regimen. Sera were collected and frozen throughout the trials for HIV-1–specific serologic assays, including neutralizing antibody assays. Sera were tested at the AVEG central immunology laboratory at Duke University for neutralizing antibody activity against the homologous vaccine strain. Several sera demonstrating activity from each trial were selected for further evaluation. A blinded panel of 51 sera, including 1 negative control from a placebo recipient, was sent to the Walter Reed Army Institute of Research laboratories for evaluation of neutralizing antibody activity against a panel of primary and laboratory-adapted HIV-1 isolates.

In addition, sera and heparinized whole blood were obtained from a convenience sample of 16 clinically asymptomatic HIV-1–seropositive volunteers attending the HIV clinic of the National Naval Medical Center (Bethesda, MD). All volunteers were HIV-1 seroconverters; the amount of time each had been infected with HIV-1 was estimated as the midpoint between the last negative and first positive HIV-1 serology. These volunteers and their sera were referred to as USI–US16. With one exception (US15), subjects had CD4+ lymphocyte counts >500/µL at the time of sample collection. The 5 primary viruses (US1–US5) used in neutralization assays were isolated from volunteers US1–US5. The sera obtained from US1–US16 were not blinded.

Virus isolation. Virus was isolated by cocultivation of ficoll-separated PBMC from HIV-1–infected subjects with phytohemagglutinin (PHA)-stimulated uninfected donor PBMC as previously described [41]. For expansion to high-titered virus stocks, culture supernatant from the initial virus isolation was used to infect 2 × 10^7 fresh PHA-stimulated donor PBMC. Cultures were followed using an EIA (Coulter, Hialeah, FL) for production of HIV-1 p24 antigen, and supernatant collected at a single time point (between days 6 and 10) was stored in 1-ml aliquots in the vapor phase of liquid nitrogen. None of these primary isolates were passaged through neoplastic cell lines. To prepare laboratory-adapted HIV strains (MN, H1B, and SF2), virus supernatant from chronically infected H9 or HUT-78 cells was used to infect human PBMC as described above.

<table>
<thead>
<tr>
<th>Serum code</th>
<th>Vaccine product</th>
<th>Dose</th>
<th>Source of antigen</th>
<th>Adjuvant or delivery system</th>
<th>Dose schedule (months)</th>
<th>Time point (weeks)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–6</td>
<td>MicroGeneSys rgp 160 IIIB</td>
<td>640 µg</td>
<td>Insect cells</td>
<td>Alum</td>
<td>0, 1, 6, 12</td>
<td>2–4 after 3rd or 4th immunization</td>
</tr>
<tr>
<td>7–11</td>
<td>Immuno rgp 160 IIIB</td>
<td>50 µg</td>
<td>Vero cells</td>
<td>Alum/DOC</td>
<td>0, 1, 6, 12</td>
<td>4 after 3rd immunization</td>
</tr>
<tr>
<td>12–16</td>
<td>Genentech rgp 120 IIIB</td>
<td>300 µg</td>
<td>CHO cells</td>
<td>Alum</td>
<td>0, 1, 6, 12</td>
<td>2 after 3rd immunization</td>
</tr>
<tr>
<td>17–21</td>
<td>Genentech rgp 120 IIIB + MN²</td>
<td>300 µg</td>
<td>CHO cells</td>
<td>Alum</td>
<td>0, 1, 8, 13, 19</td>
<td>2 after 5th immunization</td>
</tr>
<tr>
<td>22–26</td>
<td>Genentech rgp 120 IIIB + MN²</td>
<td>300 µg</td>
<td>CHO cells</td>
<td>Alum</td>
<td>0, 1, 6, 12</td>
<td>2 after 4th immunization</td>
</tr>
<tr>
<td>101–105</td>
<td>Biocine Env 2-3</td>
<td>30 µg</td>
<td>Yeast</td>
<td>MF59/MTP-PE</td>
<td>0, 1, 6</td>
<td>2–4 after 2nd or 3rd immunization³</td>
</tr>
<tr>
<td>107–112</td>
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<td>15 or 50 µg</td>
<td>CHO cells</td>
<td>MF59/MTP-PE</td>
<td>0, 1, 6</td>
<td>4 after 3rd immunization³</td>
</tr>
<tr>
<td>113–117</td>
<td>Genentech rgp 120 IIIB + MN²</td>
<td>300 µg</td>
<td>CHO cells</td>
<td>Alum</td>
<td>0, 1, 6, 12</td>
<td>2 after 3rd immunization³</td>
</tr>
<tr>
<td>119, 120, 123</td>
<td>Bristol-Myers Squibb HIVAC + rgp 120 boost⁴</td>
<td>2 HIVAC, 1 rgp 120</td>
<td>Vaccinia, CHO cells</td>
<td>Vaccinia virus, Alum, or MF59</td>
<td>0, 4, 8</td>
<td>4 after 3rd immunization³</td>
</tr>
<tr>
<td>125–129</td>
<td>United Biomedical octameric V3 peptide (MN)</td>
<td>20, 100, or Synthetic</td>
<td>MAPS</td>
<td>0, 1, 6</td>
<td>4 after 3rd immunization³</td>
<td></td>
</tr>
<tr>
<td>118</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Alum</td>
<td>0, 1, 6</td>
<td>2 after 3rd immunization³</td>
</tr>
</tbody>
</table>

NOTE. Vero cells, monkey kidney cells; DOC, deoxycholate; MF59, microfluidized oil-in-water emulsion; MTP-PE, muramyl tripeptide-phosophatidylethanolamine; MAPS, multiple antigen peptides presented on oligo-lysine backbone; rgp, recombinant envelope glycoprotein. Supplier locations: MicroGeneSys, West Haven, CT; Genentech, South San Francisco; Biocine, Emeryville, CA; Bristol-Myers Squibb, Evansville, IN; United Biomedical, Hauppauge, NY; Immuno, Vienna.

* Time sera obtained for measurement of neutralizing antibody.
³ 3 immunizations with rgp120 IIIB followed by 2 with rgp120 MN.
⁴ 500 µg each, given together.
³ Samples 104 and 105 were obtained 1 month after 2nd immunization.
⁴ HIVAC = recombinant vaccinia virus expressing rgp160 (IIIB strain). Boosts: 119, rgp120 SF2; 120 and 123, rgp120 IIIB.
**Standard neutralization assay.** Unless otherwise specified, serum neutralizing antibody activity was assessed as previously described, with minor modifications [20]. Virus growth kinetics and TCID<sub>50</sub> were determined within the assay format. The same donor cryopreserved PBMC were used in the titration and neutralization assays. To facilitate evaluation of a large number of sera, each serum sample (heat-inactivated at 56°C for 45 min) was initially assayed at a single dilution of 1:5, and selected sera were titrated by serial 5-fold dilutions.

In brief, 25 μL of test serum per well was aliquoted in quadruplicate wells of a 96-well microtiter plate. Sextuplicate wells containing pooled, heat-inactivated normal human serum (NHS) served as the control for baseline virus growth. An equal volume of virus stock (25 μL), representing 100 TCID<sub>50</sub>, was added to each well. After 30 min at 37°C, 2 × 10<sup>5</sup> PHA-stimulated PBMC was added and incubated overnight at 37°C. Cells were then washed extensively to remove p24 antigen and plasma anti-p24 antibody and transferred to a 96-well microtiter plate with culture media containing 10% interleukin-2. Inhibition of PBMC infection was assessed by quantitative p24 measurement of cell supernatants during the logarithmic growth phase (days 4–6 for viruses in this study). Average p24 antigen output in sextuplicate control (NHS) wells was usually 30–100 ng/mL (coefficients of variation range, 7%–44%; average, 18%). For sera tested at a single dilution (1:5), results were reported as percent neutralization (decrease in p24 level in test serum wells compared with NHS control wells). The final serum dilution was defined as the dilution of serum in the presence of virus (i.e., 1:10) and before addition of PBMC. To maximize sensitivity, a 50% or greater reduction in p24 antigen production was defined as a positive result.

**Infectivity reduction assay.** Selected sera were also evaluated in a neutralization assay measuring effect of serum on virus endpoint titer. Virus TCID<sub>50</sub> was determined in a microtiter plate assay using PHA-stimulated PBMC as target cells. Serial 5-fold dilutions of virus stock were preincubated in quadruplicate wells with test sera or NHS diluted 1:10. After 30 min at 37°C, 10<sup>5</sup> PHA-stimulated PBMC were added to each well. Cells were washed after 24 h and maintained with culture media containing 10% interleukin-2. Extracellular p24 antigen was measured on day 8, and wells containing >100 pg/mL were scored as positive. TCID<sub>50</sub> and 95% confidence intervals were calculated with computer software using the statistical method of Spearman-Karber [42], and the TCID<sub>50</sub> values for virus preincubated with NHS and test sera were compared. Prior experiments with multiple replicates of NHS demonstrated that TCID<sub>50</sub> values usually were within a 5-fold range, and for TCID<sub>50</sub> values differing by 10-fold, calculated 95% confidence intervals did not overlap. Thus, a TCID<sub>50</sub> reduction of >10-fold was defined as evidence of serum-mediated neutralization. Most study samples had been unblinded before the infectivity reduction assays were done.

**MT-2 phenotype assay.** The ability of primary viruses US1–US5 to induce syncytia in the MT-2 cell line was evaluated according to the AIDS Clinical Trials Group consensus protocol [43]. Syncytium-inducing viruses had ≥3 multinucleated giant cells/well.

**Results**

A total of 50 blinded serum samples from candidate vaccine recipients was evaluated for neutralizing antibody activity against 5 primary and 3 laboratory-adapted strains of HIV-1. For comparison, 16 serum samples from HIV-1–infected persons were assayed against the same viruses. Vaccinee sera were obtained from nine phase 1 clinical trials of seven different HIV-1 subunit vaccine products and one recombinant virus (vaccinia-gp160). Vaccine product, dose, adjuvant, and immunization regimen are summarized in table 1. Sera selected for the neutralization assays were from time points after the third or fourth immunization, except for the Biocine Env 2-3 product, for which 2 of 5 serum samples were collected after the second immunization time point.

Table 2 summarizes the neutralizing antibody activity of vaccinee sera against 5 primary HIV-1 isolates and 3 laboratory-adapted strains. All primary viruses were previously characterized as HIV-1 genetic subtype B by gp160 envelope gene DNA sequence (US1–US4) [20] or by heteroduplex mobility assay (US5, data not shown) [44]. The laboratory-adapted strains correspond to the 3 vaccine strains used to produce the subunit vaccine products. The results represent an average of two independent experiments (in a few cases, a third assay was done to resolve minor discrepancies). For most of the vaccine products studied, the magnitude and breadth of serum neutralizing antibodies against laboratory-adapted strains of HIV-1 have been summarized [31–37, 39, 40]. The current study was designed primarily to evaluate neutralizing antibody activity against primary HIV-1 isolates.

In brief, 25 μL of test serum per well was aliquoted in quadruplicate wells of a 96-well microtiter plate. Sextuplicate wells containing pooled, heat-inactivated normal human serum (NHS) served as the control for baseline virus growth. An equal volume of virus stock (25 μL), representing 100 TCID<sub>50</sub>, was added to each well. After 30 min at 37°C, 2 × 10<sup>5</sup> PHA-stimulated PBMC was added and incubated overnight at 37°C. Cells were then washed extensively to remove p24 antigen and plasma anti-p24 antibody and transferred to a 96-well microtiter plate with culture media containing 10% interleukin-2. Inhibition of PBMC infection was assessed by quantitative p24 measurement of cell supernatants during the logarithmic growth phase (days 4–6 for viruses in this study). Average p24 antigen output in sextuplicate control (NHS) wells was usually 30–100 ng/mL (coefficients of variation range, 7%–44%; average, 18%). For sera tested at a single dilution (1:5), results were reported as percent neutralization (decrease in p24 level in test serum wells compared with NHS control wells). The final serum dilution was defined as the dilution of serum in the presence of virus (i.e., 1:10) and before addition of PBMC. To maximize sensitivity, a 50% or greater reduction in p24 antigen production was defined as a positive result.

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Results in table 2 confirm that immunization with each of the vaccine products elicited serum neutralizing antibodies against the homologous HIV-1 vaccine strain. Of 50 sera, 49 neutralized at least 1 of the laboratory-adapted HIV-1 strains and in many cases, sera also neutralized heterologous laboratory-adapted strains. At the 1:10 dilution, some vaccinee sera essentially prevented infection of PBMC by 100 TCID<sub>50</sub> of the homologous virus (>99% neutralization; figure 1). Several of these strong neutralizing sera were titered by serial 5-fold dilutions against the 3 laboratory-adapted strains and were compared with serum from an HIV-1–infected subject (US2) that has strong neutralizing activity against primary HIV-1 isolates [20]. Neutralization curves of serum from US2 and selected vaccinee sera were similar against prototype strains, especially MN and SF2 (figure 1). However, despite levels of neutralizing antibody against laboratory-adapted strains that were occasionally similar to those induced by natural HIV-1 infection, none of the vaccinee sera demonstrated neutralizing activity against any of the 5 primary isolates of HIV-1 (table 2, figure 1). Thus, antisera elicited by all of the vaccine products could neutralize laboratory-adapted but not primary isolates of HIV-1. The MT-2 cell phenotype of the primary viruses did not affect this result. Of the 5 viruses, 2 (US1 and US2) were syncytia-inducing and 3 were non–syncytia-inducing in the MT-2 phenotype assay.

In contrast to sera from the vaccine recipients, sera from clinically asymptomatic HIV-1–infected persons could often significantly neutralize primary isolates (table 3, figure 1).
a 1:10 dilution, neutralization values for the 16 sera against the 5 primary isolates ranged from 4% to 99%, and each of the 5 viruses was strongly neutralized (>90%) by at least several sera. Of note, the average neutralization for the 16 sera against each of the 3 laboratory-adapted strains ranged from 93% to 98% compared with 64%–78% against the 5 primary viruses. These data are consistent with those from earlier reports showing primary HIV-1 isolates to be less susceptible than laboratory strains to neutralizing antibody yet fully susceptible to neutralization by sera from some HIV-1-infected persons [19, 20, 45].

Primary isolates are also relatively resistant to neutralization by sCD4, although this resistance can be overcome by prolonged preincubation of virus and sCD4 at 4°C [46, 47]. Thus, using primary isolates US1 and US2 and 5 vaccinee sera with strong neutralizing activity against laboratory strains (samples 10, 22, 24, 108, and 110), sera and virus were preincubated for 8 h at 4°C prior to addition of cells. Using these same 2 viruses and 5 sera, several other experimental modifications of the standard neutralization assay were done. To test for a possible prozone effect, vaccinee sera were evaluated undiluted and diluted 1:10, 1:100, and 1:1000. To assess the influence of complement proteins, aliquots of these sera, which had never been heat-inactivated, were again tested at dilutions of 1:10, 1:100, and 1:1000. Results of each of these experiments were similar: Viruses US2 and US4 were neutralized with antisera from HIV-1-infected subjects but not with antisera from envelope subunit vaccine recipients (data not shown).

In the previous experiments, neutralization assays were done with a high (100 TCID₅₀) virus inoculum. To assess the possibility that vaccinee sera would demonstrate neutralizing activity at a lower virus inoculum, selected sera were studied in an infectivity reduction assay. As described in Methods, a TCID₅₀ reduction of 10-fold or greater by test sera (compared with NHS) was defined as evidence of serum-mediated neutralization. Two strong neutralizing serum samples from HIV-1-infected volunteers and 8 selected samples from vaccine recipients were tested against viruses US1 and US2. The vaccinee serum samples each strongly neutralized the homologous vaccine strain (table 4). Virus TCID₅₀ was not significantly altered by preincubation with sera from vaccine recipients. In contrast, serum samples from HIV-1-infected subjects US12 and US16 reduced virus TCID₅₀ by 17- to 84-fold. For vaccinee serum sample 119, a repeat experiment using virus US1 again showed a nonsignificant reduction (2-fold) in virus titer compared with that in the control.

**Discussion**

Our data confirm that immunization with HIV-1 envelope subunit candidate vaccines can elicit high levels of functional (neutralizing) antibodies against laboratory-adapted strains of HIV-1. In a few selected cases, the level was similar in magnitude to that found in natural infection (figure 1). Yet, none of the sera tested had detectable neutralizing activity against 5 primary HIV-1 isolates. This result was obtained in two different neutralization assays and persisted despite attempts to increase assay sensitivity. Others have similarly reported that sera from HIV-1 subunit vaccine recipients displayed neutralizing activity against prototype but not primary isolates of HIV-1 [24, 48, 49]. The current study was not designed to compare the magnitude of neutralization elicited by each product against
laboratory-adapted strains; thus, for most sera, neutralization of prototype strains was assessed at only a 1:10 serum dilution, and conclusions based on the percent neutralization at a single dilution are limited.

As previously mentioned, primary isolates are markedly less sensitive to neutralization by sCD4 and polyclonal antibody than are laboratory-adapted strains. Nonetheless, each of the 5 primary isolates evaluated was neutralized by some of the sera from HIV-1-infected subjects in our panel. Several of the stronger neutralizing sera exhibited 50% titers in the range of 1:100–1:800 against primary isolates [20] (data not shown) and displayed 90%–99% neutralization at a 1:10 dilution (table 3). This contrasts with the inability of even undiluted vaccinee sera to inhibit p24 antigen production by 50%. It is possible that vaccinee sera contain low levels of primary isolate neutralizing antibodies that were undetectable in our assays. However, if the difference in the neutralizing antibody response induced by subunit vaccination and natural infection is quantitatively different, it appears to be large in magnitude.

The well-described disparity in neutralization sensitivity between laboratory-adapted and primary isolates suggests fundamental differences in neutralization epitopes or their accessibil-
Table 3. Neutralization of primary isolates by sera from HIV-1–infected persons.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>US1</td>
<td>24</td>
<td>68</td>
<td>89</td>
<td>90</td>
<td>90</td>
<td>79</td>
<td>87</td>
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<td>35</td>
<td>97</td>
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</table>

NOTE. US1–US16 are 16 clinically asymptomatic HIV-1–infected persons. Values represent % reduction in p24 expression by phytohemagglutinin-stimulated peripheral blood mononuclear cells (as described in Materials and Methods). Strong neutralizing activity (=90% neutralization) is indicated in bold.

* Estimated no. of months subject infected (as described in Materials and Methods).
† Arithmetic mean of % neutralization values for each serum against 5 viruses.
‡ Neutralization with autologous serum obtained at same time virus was isolated. This result was omitted from calculated average % neutralization value for each serum.

It is possible that immunization with current HIV-1 envelope subunit vaccine products does not elicit antibodies to epitopes that are essential for the neutralization of primary isolates. Others have demonstrated that the V3 region of gp120 is the principal neutralizing determinant for laboratory-adapted strains [11, 32, 52]. In serum from envelope subunit vaccine recipients, the presence of fairly high levels of neutralizing antibodies directed against the V3 region [32, 53,

Table 4. HIV-1 infectivity reduction assay results.

<table>
<thead>
<tr>
<th>Serum code no.</th>
<th>Vaccine product vs. (vaccine strain)*</th>
<th>TCID50</th>
<th>Infectivity reduction</th>
<th>TCID50</th>
<th>Infectivity reduction</th>
</tr>
</thead>
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<tr>
<td>NHS pool</td>
<td>---</td>
<td>3125</td>
<td>1</td>
<td>2090</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Immuno rgp 160 (IIIB)</td>
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<td>1</td>
<td>2090</td>
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</tr>
<tr>
<td>23</td>
<td>Genentech rgp 120 (IIIB + MN)</td>
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<td>1</td>
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<td>25</td>
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<td>128</td>
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<td>--- (alum)</td>
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<tr>
<td>US12</td>
<td>HIV-1 infected</td>
<td>98 (MN)</td>
<td>84</td>
<td>37</td>
<td>56</td>
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<tr>
<td>US16</td>
<td>HIV-1 infected</td>
<td>99 (MN)</td>
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<td>125</td>
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NOTE. End-point titer (TCID50) for virus preincubated with pooled normal human sera (NHS) was compared with TCID50 for virus preincubated with test sera. Infectivity reduction is virus TCID50 in NHS/TCID50 in test serum. Data are results from 1 set of experiments. See table 1 for supplier locations.

* % reduction in p24 antigen at 1:10 serum dilution (as described in Materials and Methods for standard neutralization assay).
only about one-third of the sera would be active against each neutralizing activity [19, 21, 45, 57]. Neutralizing activity against the 5 primary isolates. However, the absence of a detectable neutralizing antibody response could be elicited in vaccinee sera are completely deficient in antibodies with functional neutralization against the 5 primary isolates were from persons infected for ~18 months (US6, US7, and US10; table 3). In contrast, the 5 sera demonstrating 90% or greater neutralization against the primary isolates were from persons infected for ~5–7 years (US2, US4, US5, US12, and US16). While this study was not designed to correlate time of HIV-1 infection with serum neutralizing antibody activity, these data suggest a general trend of low neutralizing antibody levels early in HIV infection, with an increase in magnitude and breadth over several years in asymptomatic persons. Data in table 3 indicate that most of the sera from HIV-1–infected subjects had 50% neutralizing activity against the 5 primary isolates. However, if a more stringent criterion of 90% neutralization were used, only about one-third of the sera would be active against each virus. This is consistent with the results of others, which indicate that not all sera from HIV-1–infected persons have strong neutralizing activity [19, 21, 45, 57].

Our data do not exclude the possibility that vaccinee sera contain low levels of neutralizing antibody against primary HIV-1 isolates. Assays for functional antibody may not be particularly sensitive [58–61], and it is possible that serum neutralizing activity was below the level of detection in our assays. Our limited data showing that neutralizing activity of sera is weak during the first 18 months of infection are consistent with those from several prior studies showing that neutralizing antibodies are often not detectable for weeks to months after acute HIV-1 infection [57, 62, 63]. In contrast, gp120-binding antibodies can be detected within several days of clinical presentation with acute HIV infection [63]. While the cellular arm of the immune system may be completely responsible for abrogating the high-level plasma viremia of acute infection [64], it is equally possible that undetectable levels of functional antibody play a role. As in primary infection, the absence of measurable neutralizing antibody does not prove that the vaccinee sera are completely deficient in antibodies with functional activity against primary viruses. Since the magnitude and breadth of the neutralizing antibody response seem to increase over time in natural HIV-1 infection, it is feasible that a detectable neutralizing antibody response could be elicited in vaccinees with booster immunizations over time or with better adjuvants and immunization regimens.

As currently formulated, HIV-1 envelope subunit immunogens clearly elicit a lesser magnitude and breadth of humoral immunity than those found in natural HIV-1 infection. In the absence of established correlates of protection for HIV-1, it is reasonable and rational to attempt to improve the functional humoral responses induced by these vaccine products. As we do not know what aspects of the humoral and cellular immune response will confer protection against HIV-1 infection or disease, the inability to detect neutralizing antibodies to primary viruses in this in vitro assay system should not be presumed to predict the ultimate success or failure of envelope subunit products in human phase III clinical trials. Indeed, there are several examples of effective retroviral vaccines that do not elicit detectable levels of neutralizing antibodies [65–67]. The validation of any postulated in vitro correlates of protection for HIV-1 must await in vivo studies showing vaccine efficacy.

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