Control of Viremia and Prevention of Simian-Human Immunodeficiency Virus-Induced Disease in Rhesus Macaques Immunized with Recombinant Vaccinia Viruses plus Inactivated Simian Immunodeficiency Virus and Human Immunodeficiency Virus Type 1 Particles

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An effective vaccine against the human immunodeficiency virus type 1 (HIV-1) will very likely have to elicit both cellular and humoral immune responses to control HIV-1 strains of diverse geographic and genetic origins. We have utilized a pathogenic chimeric simian-human immunodeficiency virus (SHIV) rhesus macaque animal model system to evaluate the protective efficacy of a vaccine regimen that uses recombinant vaccinia viruses expressing simian immunodeficiency virus (SIV) and HIV-1 structural proteins in combination with intact inactivated SIV and HIV-1 particles. Following virus challenge, control animals experienced a rapid and complete loss of CD4+ T cells, sustained high viral loads, and developed clinical disease by 17 to 21 weeks. Although all of the vaccinated monkeys became infected, they displayed reduced postpeak viremia, had no significant loss of CD4+ T cells, and have remained healthy for more than 15 months postinfection. CD8+ T-cell and neutralizing antibody responses in vaccinated animals following challenge were demonstrable. Despite the control of disease, virus was readily isolated from the circulating peripheral blood mononuclear cells of all vaccinees at 22 weeks postchallenge, indicating that immunologic control was incomplete. Virus recovered from the animal with the lowest postchallenge viremia generated high virus loads and an irreversible loss of CD4+ T-cell loss following its inoculation into a naïve animal. These results indicate that despite the protection from SHIV-induced disease, the vaccinated animals still harbored replication-competent and pathogenic virus.

Prophylactic vaccines that prevent diseases caused by viral pathogens typically elicit neutralizing antibodies (Abs), which rapidly clear virus, and/or cellular immune responses, which eliminate virus-producing cells. In a well-studied prototypical mouse retroviral system, both arms of the immune system are needed to control Friend murine leukemia virus-induced viremia and/or splenomegaly and to prevent the transition to a persistent infection status that invariably leads to erythroleukemia and death (35). Because vaccines directed against the human immunodeficiency virus type 1 (HIV-1) have failed to elicit Abs that neutralize primary HIV-1 strains of diverse geographic and/or genetic origins (5, 9, 31, 47, 51, 77), recent efforts have primarily focused on regimens that stimulate cell-mediated immunity. This retargeting of the vaccine effort towards cellular immune responses is based on numerous reports showing that cytotoxic T lymphocytes (CTL) play a major role in controlling both acute and chronic HIV-1 infections (13, 43, 52, 54, 64). A direct demonstration of this effect in an animal model comes from experiments showing that depletion of rhesus macaque (Macaca mulatta) CD8+ T cells with monoclonal Abs (MAbs) at the time of primary simian immunodeficiency virus (SIV) and chimeric simian-human immunodeficiency virus (SHIV) infections leads to markedly elevated levels of viremia at the peak of the acute infection and more rapid onset of disease (38, 48, 67).

A variety of vaccine regimens, designed to stimulate antiviral cellular immune responses, control to various degrees SIV and SHIV infections. These include immunizations with plasmid DNAs and live viral vectors, used singly or in combination (2, 8, 14, 21, 24, 32, 41, 65, 69, 74). While transient reductions in the postpeak viremia (virus set point) were achieved for some vaccinated macaques challenged with pathogenic SIVmac strains (33, 55), more-impressive control of virus replication and prevention of the rapid and irreversible depletion of CD4+ T cells have been obtained in studies that utilized the highly pathogenic SHIV-89.6P strain (2, 7, 8, 18, 65, 74). However, the vaccine-elicited CTL responses only modestly reduced virus replication during the first 2 to 3 weeks of infection in both SIV- and SHIV-challenged animals and, in the case of the SIV-infected monkeys, failed to prevent the development of
immunodeficiency in many of the vaccinees (1, 10, 33, 45, 55, 56, 60).

There are now several reports showing that neutralizing Abs directed against HIV-1 envelope proteins can effectively block infections both in vitro and in vivo: monoclonal and polyclonal neutralizing Abs passively administered to animals prior to virus challenge have, in fact, been shown to confer sterilizing protection (25, 30, 46, 57, 71). The b12 MAb, which binds to a gp120 epitope that includes the CD4 interaction site and can neutralize multiple primary HIV-1 isolates in vitro (16), completely protects rhesus monkeys against a vaginal challenge neutralizing activity against a wide range of primary HIV-1 isolates.

As a trimeric complex on virus particles, HIV-1 gp120 contains at least three potential targets for neutralization: (i) a shielded and small highly conserved surface containing the binding sites for the CD4 and chemokine receptors, (ii) external variable loops (predominantly V1-V2 and V3) that are unique (type specific) for each virus isolate, and (iii) a heavily glycosylated face, present on the exterior surface of each gp120/gp41 heterodimer (59). Several strategies have been proposed (and in some cases, tested) to induce potent neutralizing Abs against a wide range of primary virus isolates. Some investigators have suggested eliminating glycosylation sites (63) or removing variable loops (17, 76, 81, 82), which occlude extensive portions of the conserved neutralization surface of gp120, as strategies for increasing the breadth of the humoral response elicited by HIV-1 Env preparations. We have immunized macaques with a mixture of several different envelopes (recombinant vaccinia virus prime-protein boost) to potentially broaden the humoral response. However, this approach elicited neutralizing Abs only against virus strains included as immunogens in the vaccine (19). Infection was prevented only in those animals challenged with a SHIV possessing an envelope homologous to one present in the immunizing antigen mix but not in animals exposed to virus bearing a heterologous envelope glycoprotein.

Because immunizations with monomeric gp120 elicit Abs that preferentially bind to denatured Env preparations and generate only type-specific neutralizing Abs (5, 9, 15, 20, 31, 47, 80), soluble oligomeric envelope immunogens have been constructed in an effort to mimic the conformation of gp120/gp41 present on virus particles and potentially increasing the effectiveness of the immune response. Toward this end, truncated HIV-1 envelope glycoproteins containing (i) a mutated gp120/gp41 proteolytic cleavage site, (ii) the introduction of novel cysteine residues for the formation of intersubunit disulfide bonds, or (iii) the addition of GCN4 trimer helices to stabilize the gp120/gp41 heterodimer have been prepared (12, 22, 28, 83). Immunization of rhesus monkeys with one of these preparations elicited modest neutralization against heterologous laboratory-adapted HIV-1 strains but no demonstrable neutralizing activity against primary virus isolates (23).

In this study we have utilized the pathogenic SHIVgp120r- rhesus macaque animal model system (26, 37) to evaluate a vaccine regimen, which presents the HIV-1 envelope in its most native form, i.e., as a component of intact virions. Aldri-thiol-2 (AT-2)-inactivated particles were administered to boost immune responses initially primed with a mixture of recombinant vaccinia viruses (rVVVs) expressing SIV Gag and Pol proteins and HIV-1 Env. AT-2 was used for inactivation because previous studies had shown that this treatment preserved the native structure and function of virion-associated envelope proteins (66). Inactivated SIV particles (3) were also included in the immunization mix to elicit immune responses against several other viral proteins (viz., Gag and Pol). Following challenge with the closely related but heterologous (relative to the HIV-1 Env antigens) SHIVgp120r-PS1, vaccinated animals readily controlled virus infection and SHIV-induced depletion of CD4+ T lymphocytes. CD8+ T-cell and neutralizing-Ab responses were detected following challenge, suggesting that cellular and humoral immunity contributed to the protection conferred by the vaccine regimen. Despite the control of SHIV-induced disease, replication-competent virus was isolated from all four vaccinated animals 22 weeks postchallenge. The virus recovered from the vaccinated monkey with the lowest set point viremia caused a rapid and irreversible loss of CD4+ T cells following inoculation into a naive animal. These results demonstrate that an ongoing infection by a highly pathogenic SHIV was being controlled by the immune system of the vaccinated rhesus macaques.

**MATERIALS AND METHODS**

**Cells and viruses.** MT4 cells (34) were propagated in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Rhesus macaque peripheral blood mononuclear cells (PBMC) were prepared from EDTA-treated whole blood by Ficoll-Paque density gradient centrifugation, stimulated in RPMI-FBS medium containing 25 μg of concanavalin-A/ml for 4 h, and then cultured for an additional 48 h in RPMI-FBS medium containing 20 U of interleukin-2 (IL-2)/ml prior to their use as previously described (37).

The construction of the nonpathogenic SHIVDH12r has been previously reported (72). The highly pathogenic SHIVDH12r was isolated at week 68 from rhesus monkey 565Z, which had been treated with an anti-human CD8 MAb at the time of its primary infection with the nonpathogenic SHIVDH12r (37). Virus isolated at week 52 from animal 565Z also induced an irreversible and extremely rapid depletion of CD4+ T lymphocytes following its inoculation into rhesus monkey PS1 and was designated SHIVDH12r-PS1 (Fig. 1). A tissue culture stock of SHIVDH12r-PS1 was prepared by cocultivating a mixture of PBMC and a lymphoid tissue suspension, recovered at the time of necropsy of macaque PS1, with mitogen-activated PBMC from a naive rhesus monkey. The titers of both SHIVDH12r (used for immunoblotting and virus neutralization assays) and SHIVDH12r-PS1 (used as the challenge virus and in neutralization assays) were measured by end point dilution in rhesus PBMC and MT-4 cells. The TCID50 for each virus stock was calculated by the method of Reed and Muench (61).

rVVVs and inactivated SIV and HIV-1 virions. The construction and use of rVVVs that express the HIV-1 envelope in its most native form, i.e., as a component of intact virions. The amount of the virus capsid (CA) protein present in the final suspension was determined by antigen capture. Microvesicles, which are released from CEM X174(T1) cells and copurify with virions (11), were prepared from uninfected cells and similarly treated with AT-2.
Vaccination of rhesus monkeys. The six rhesus monkeys used in this study were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care standards. Animals were anesthetized prior to phlebotomy, immunizations, and virus challenge as previously described (37). Four monkeys were initially vaccinated with a mixture of vvDHenv and vvSIVag-pol (5 × 107 PFU of each virus) at weeks 0 and 8 by intradermal injection (four sites per animal). On weeks 20 and 28, each animal received intramuscular injections (two sites on each leg) of the AT-2-treated HIV-1 DH12 (16.5 ng of p24) and SIVmac239 (12 μg of p27) virion mixture in combination with 100 μg of the QS21 adjuvant (Aquila Biopharmaceuticals, Framingham, Mass.). The total amount of protein (viral plus cellular) in each virion boost was approximately 2.5 mg. The two control animals each received intradermal inoculations of the control vaccinia virus recombinant vTF7-3 (108 PFU) followed by intramuscular injections of QS21. Additional 6 days. Culture supernatants were assayed for reverse transcriptase (RT) activity to detect the presence of replicating virus (79). For a given sample, the absence of RT activity in all replicate wells was scored as virus neutralization. In some cases, plasma samples were serially diluted to determine the antivirus endpoint neutralizing Ab titer, which was calculated by the method of Reed and Muench.

Animal challenge, virus loads, and in vivo lymphocyte analyses. All animals were challenged intravenously with 500 TCID₅₀ (based on the titer in MT4 cells) of the highly pathogenic SHIV_DH12R-PS₁ 18 weeks following the final immunization. End point titrations of the SHIV_DH12R-PS₁ challenge virus stock in naïve rhesus macaques revealed that this inoculum size consistently resulted in the rapid and irreversible depletion of CD4⁺ T lymphocytes to <20 cells/µl of plasma, high virus loads, and the development of clinical disease (Y. Endo and R. Willey, unpublished data).

Viral RNA levels in plasma were determined by real-time PCR using reverse-transcribed viral RNA templates extracted from EDTA-treated blood samples as previously reported (44).

Lymphocyte subsets were analyzed by staining EDTA-treated blood samples with fluorochrome-conjugated MAbs (anti-CD3, anti-CD4, anti-CD8, and anti-CD20) and analyzed by flow cytometry (FACSort; Becton Dickinson) as described previously (37). Isolation of virus from vaccinated monkeys and subsequent animal challenge. Virus isolates were obtained from all four vaccinated animals 22 weeks after challenge. PBMC were depleted for CD8⁺ T lymphocytes by using anti-CD8 magnetic beads (Miltenyi Biotec GmbH). The remaining cells were stimulated with 25 μg of concanavalin A/ml for 24 h, and after the mitogen containing medium was removed, the CD4⁺-enriched cells (4.0 × 10⁶) were cocultured with freshly prepared and mitogen-activated PBMC (1.5 × 10⁶) from uninfected rhesus monkeys. Culture supernatants were collected every 2 to 3 days, and peak RT-containing samples were aliquoted and frozen at −70°C. The virus recovered from animal BF16 (10⁵ TCID₅₀) was inoculated intravenously into a naïve rhesus monkey, and viral RNA and CD4⁺ T-cell levels in its peripheral blood were monitored following challenge.

In vivo CD8⁺ T-cell depletions. The anti-human CD8 MAb OKT8/F (kindly provided by K. Demarest and T. Mercolino, The R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.) was administered to animals BF08 and BF16 at 27 weeks following SHIV_DH12R-PS₁ challenge. On the basis of preliminary dose-response experiments (T. Igarashi and M. Martin, unpublished data), a single intravenous injection consisting of 1 mg of the MAAb/kg of body weight was administered. Blood was collected from each animal on days 1 to 4, 7, 9, and 11 to determine virus loads and lymphocyte subset levels.
RESULTS

Vaccinations and immune responses. The vaccine protocol and immunization schedule followed are shown in Fig. 2. The four animals constituting the vaccine group were immunized by the intradermal route at weeks 0 and 8 with rVV s expressing the SIV Gag/Pol and HIV-1 Env proteins followed by intramuscular injections with AT-2-inactivated SIV and HIV-1 virions (weeks 20 and 28). Two control animals received rVV expressing the T7 polymerase followed by AT-2-treated microvesicles at the same time intervals. All animals were challenged with SHIVDH12R-PS1 18 weeks following the last immunization.

![FIG. 2. Vaccine study groups, immunogens, and schedule. Viral antigen-vaccinated animals (n = 4) were immunized twice (weeks 0 and 8) with rVV s expressing the SIV Gag/Pol and HIV-1 Env proteins followed by intramuscular injections with AT-2-inactivated SIV and HIV-1 virions (weeks 20 and 28). Two control animals received rVV expressing the T7 polymerase followed by AT-2-treated microvesicles at the same time intervals. All animals were challenged with SHIVDH12R-PS1 18 weeks following the last immunization.](image)

The reactivity of HIV-1- and SIV-specific Abs in plasma collected from the immunized animals was evaluated by immunoblotting. Viral proteins present in a lysate of pelleted SHIVDH12 particles, to which HIV-1DH12 gp120 (previously purified from rVV-infected HeLa cells) was added, were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to membranes. An immunoblot of plasma reactivity from a representative vaccinated animal (macaque BF08) revealed no binding to virus-specific proteins following either of the rVV vaccinations (Fig. 3, upper panel). In contrast, Abs directed against HIV-1 gp120 and SIV p27 CA became detectable within 2 weeks of immunization with the mixture of AT-2-inactivated HIV-1 and SIV particles. The durability of this Ab response was short-lived, falling to very low levels within a few weeks of vaccination. Virus-specific Abs were not detected in the immunoblots from the control animals: the two prominent reactive bands in the 65- to 90-kDa range observed with plasma samples from control monkey BD83 following the second microsphere boost did not correspond in size to authentic SIV or HIV gene products (Fig. 3, lower panel) and presumably represented binding to cellular proteins present in the virion lysate-gp120 mixture. While the reactivity profiles varied somewhat among the four vaccinated animals, all of these monkeys, except BF16, produced Abs against Gag and Env antigens following the final AT-2 virion immunization (Table 1). Animal BF16 generated a weak response to SIV p27 CA, but only after the second boosting with inactivated SIV and HIV-1 particles.

Since the immunizations had successfully induced virus-specific humoral responses, selected plasma samples were screened for the presence of neutralizing Abs. We have previously described and extensively used an assay that measures 100% neutralization against HIV-1 and SHIVDH12 (73, 78). Neutralizing activity against SHIVDH12, which carries an HIV-1 gp120 envelope protein that is identical to that used for immunization, was assessed by measuring virion-associated RT activity released from rhesus PBMC into the supernatant medium 12 days postinfection. Samples of macaque plasma were initially screened at a 1:10 dilution for neutralization against 100 TCID50 of SHIVDH12, also prepared in rhesus monkey
TABLE 1. SIV- and/or HIV-1-specific immunoblot responses elicited prechallenge

| Animal       | rVV | AT-2-treated microvesicles or virionsa | Post-round 1 | Post-round 2 | Post-round 1 | Post-round 2 |
|--------------|-----|--------------------------------------|--------------|--------------|--------------|--------------|
| Control macaques |     |                                      |              |              |              |              |
| BD83         | ND  | ND                                   | ND           | ND           | ND           | ND           |
| BD89         | ND  | ND                                   | ND           | ND           | ND           | ND           |
| Vaccinated macaques |     |                                      |              |              |              |              |
| BF08         | ND  | ND                                   | Gag/Env      | Gag/Env      | ND           | ND           |
| BF16         | ND  | ND                                   | ND           | Gag          | ND           | ND           |
| BF17         | ND  | Env                                  | Gag/Env      | Gag/Env      | ND           | ND           |
| BF35         | ND  | Env                                  | Gag/Env      | Gag/Env      | ND           | ND           |

a Control macaques were immunized with microvesicles, and vaccinated macaques were immunized with virions.

b ND, none detected.

PBMC. As expected, the plasma from control animals BD83 and BD89 contained no neutralizing activity against SHIVDH12 (Table 2). Neutralizing Abs were detected in one vaccinated monkey (BF35) following the second rVV immunization, and only after the first HIV-SIV virion immunizations in animals BF08 and BF17. Titers of neutralizing Ab levels against SHIVDH12 were determined by end point dilution by using plasma samples collected 2 weeks following the final AT-2-inactivated particle boost. As indicated in Table 2, the anti-SHIVDH12 titers ranged from 1:40 to 1:113 for three of the four vaccinated macaques. No neutralizing Abs were detected for immunized animal BF16, the same monkey that produced only low levels of binding Ab against SIV p27 Gag following the second vaccination with inactivated HIV and SIV particles (Table 1).

Since SHIVDH12R-PS1, an uncloned highly pathogenic derivative of SHIVDH12, which bears a gp120 containing multiple amino acid substitutions compared to the gp120 of SHIVDH12 (37), was to be used as challenge virus in these animals, its sensitivity to neutralization was also determined. No anti-SHIVDH12R-PS1 neutralizing activity was detected in any of the plasma samples collected from vaccinated or control monkeys. Thus, immunization with inactivated virions was successful in eliciting only type-specific neutralizing Ab; the presence of less than 20 amino acid substitutions in the envelope glycoprotein had rendered the virus that was to be used for challenge, SHIVDH12R-PS1, neutralization resistant. This result is consistent with our previously reported failure to measure neutralizing Abs against SHIVDH12R in monkeys chronically infected with the closely related SHIVDH12 (37; T. Igarashi, unpublished data) or to detect anti-SHIVDH12R neutralizing activity in IgG prepared from chimpanzees persistently infected with HIV-1DH12 (T. Igarashi, Y. Nishimura, and M. A. Martin, unpublished data).

TABLE 2. Anti-SHIVDH12 neutralizing-Ab titers elicited prechallenge

| Animal       | rVV | AT-2-treated microvesicles or virionsa | Post-round 1 | Post-round 2 | Post-round 1 | Post-round 2 |
|--------------|-----|--------------------------------------|--------------|--------------|--------------|--------------|
| Control macaques |     |                                      |              |              |              |              |
| BD83         | ND  | ND                                   | ND           | ND           | ND           | ND           |
| BD89         | ND  | ND                                   | ND           | ND           | ND           | ND           |
| Vaccinated macaques |     |                                      |              |              |              |              |
| BF08         | ND  | ND                                   | 1:10         | 1:40         | ND           | ND           |
| BF16         | ND  | ND                                   | ND           | ND           | ND           | ND           |
| BF17         | ND  | ND                                   | 1:10         | 1:113        | ND           | ND           |
| BF35         | ND  | ND                                   | 1:10         | 1:63         | ND           | ND           |

a Plasma samples were diluted 1:10 in the neutralization assays conducted in both rounds following rVV immunization and after round 1 following microvesicle or virion immunization. The samples collected after round 2, following microvesicle or virion immunization, were serially diluted, and the neutralizing-Ab titers were calculated by the method of Reed and Muench (60). ND, none detected.

b Control macaques were immunized with microvesicles, and vaccinated macaques were immunized with virions.

Frozen viable PBMC collected from the vaccinated animals were screened for cellular immune responses by enzyme-linked immunospot assay, using overlapping 15-mer peptides encompassing the SIV Gag-coding region. No significant increase above the background medium response was observed in cells analyzed 2 weeks following the final immunizations (data not shown).

Challenge of rhesus monkeys with SHIVDH12R-PS1. Eighteen weeks after the final immunization with the mixture of SIV and HIV-1 particles, all six animals were intravenously inoculated with 500 TCID50 of the highly pathogenic SHIVDH12R-PS1. The two control macaques, BD83 and BD89, developed peak virus loads of \(2 \times 10^8\) and \(7 \times 10^7\) viral RNA copies/ml, respectively, at weeks 2 and 3 postchallenge, which was accompanied by a rapid and irreversible loss of CD4+ T cells in both animals (Fig. 4A and B). This is the signature response associated with acute SHIVDH12R infections of naïve rhesus monkeys following intravenous virus inoculation (26, 37). Although the plasma viremia in the two control animals declined 20- to 90-fold over the next several weeks, virus production subsequently increased to very high levels and both macaques had to be euthanatized at weeks 17 and 21 because of intractable diarrhea and marked weight loss.

SHIVDH12R-PS1 challenge of the vaccinated rhesus monkeys also resulted in the rapid appearance of plasma viremia in all four animals (Fig. 4C). For three of these macaques, peak virus loads ranged between \(1.8 \times 10^4\) and \(2.8 \times 10^6\) viral RNA copies/ml at weeks 2 to 3 postinoculation, while for the fourth macaque (BF17) the viremia was somewhat higher (\(1.6 \times 10^7\) RNA copies/ml). In contrast to the control animals, viral RNA levels in the plasma of the four vaccinated macaques steadily declined over the next several months; at week 20, the SHIV RNA copy numbers per milliliter ranged from 290 to 2,100 in monkeys BF08, BF17, and BF35 and below the limit of detection (100 copies/ml) in monkey BF16. Two of the vaccinated animals experienced modest CD4+ T-cell decline within 1 to 2 weeks of virus challenge. The sharpest drop occurred for monkey B17, which had sustained the highest virus load in plasma postchallenge (Fig. 4D). The number of circulating CD4+ T cells of this macaque subsequently returned to prechallenge levels.
Postchallenge antivirus neutralizing Ab responses. The absence of a rapid and irreversible CD4\(^+\) T-lymphocyte depletion coupled with markedly restricted virus replication in the four vaccinated animals suggested that the immunization protocol had elicited a protective immune response(s). Although no anti-SHIV\(_{DH12R-PS1}\) neutralizing Abs were demonstrable prior to challenge, plasma samples collected at various times postchallenge were screened for the possible emergence of virus-neutralizing activity. As shown in Table 3, by week 3 postchallenge, all of the vaccinated animals were producing substantial titers of neutralizing Ab directed against SHIV\(_{DH12R-PS1}\). End point titration of the week 3 plasma samples revealed that virus-neutralizing Ab levels varied greatly among the vaccinated monkeys, with macaque BF16 having the highest (1:1,015) and BF08 the lowest (1:66) titers. The vaccinated animals continued to generate anti-SHIV\(_{DH12R-PS1}\) neutralizing Abs between weeks 4 and 10 (data not shown), although end point titrations were not carried out for these samples. By week 12 postinfection, neutralizing Ab titers had increased for monkeys BF17 (1:320) and BF35 (1:320), decreased for animal BF16 (from 1:1,015 to 1:272), and remained the same for BF08 (1:63). The decline in the neutralization titer measured for animal BF16 may have reflected the rapidly falling virus loads in plasma that occurred over the initial 10 to 12 weeks postchallenge (Fig. 4). In contrast, persistent virus replication in macaques BF17 and BF35 may have stimulated increased levels of anti-SHIV\(_{DH12R-PS1}\) neutralizing Abs in both monkeys.

Characterization of the SHIV persisting in the vaccinated animals. The rapid control of viremia after the SHIV\(_{DH12R-PS1}\) challenge observed for the four immunized macaques is consistent with other studies reporting that prime and boost vaccine regimens frequently confer protection against another highly pathogenic SHIV, SHIV\(_{89.6P}\) (62). Given that SHIV\(_{DH12R-PS1}\) and SHIV\(_{89.6P}\) stocks are both uncloned and that low virus concentrations are commonly used to challenge animals in vaccine experiments, the possibility exists that the virus circulating in monkeys protected by prime and boost immunization protocols may exhibit altered antigenic and
The persistently replicating SHIV had undergone sufficient antigenic change(s) to escape neutralization by Abs still capable of suppressing the input SHIVDH12R-PS1.

The pathogenic properties of SHIVs persisting at low levels in healthy vaccinated monkeys were assessed by inoculating a naïve animal (VVF) with virus recovered from one of the immunized macaques. For this purpose, the SHIV isolated from monkey BF16 at week 22 was selected because this animal had mounted the most effective suppression of virus after challenge (Fig. 4) and had experienced no discernible change in CD4+ T-cell levels. Following the inoculation of virus recovered from the vaccinated monkey, macaque VVF suffered the rapid and irreversible decline of CD4+ T lymphocytes typically observed during acute infections with SHIVDH12R-PS1 (Fig. 5). By week 3, the virus load in the blood had peaked at 10^8 RNA copies/ml and the CD4+ T-cell number had fallen to 21 cells/µl. This animal was euthanatized at week 25 because of its deteriorating clinical condition. Thus, although vaccination had prevented the invariably fatal SHIVDH12R-PS1-induced immunodeficiency, monkey BF16 remained persistently infected with a highly pathogenic virus.

Depletion of CD8+ T lymphocytes perturbs the control of viremia in vaccinated monkeys. Several previous studies have shown that vaccination of macaques with rVVas expressing SIV and/or HIV-1 proteins elicits potent cell-mediated immune responses both prior to and following virus challenge (2, 7, 33, 68, 69). To directly assess the role of cellular responses in controlling viremia, the anti-human CD8 MAb, OKT8F, was administered intravenously to rhesus monkeys BF08 and BF16 27 weeks following challenge with SHIVDH12R-PS1. Because it had been previously reported that OKT8F-induced depletion of CD8+ T lymphocytes was accompanied by the nonspecific loss of CD4+ T and CD20+ B lymphocytes in some treated macaques (39, 50), we elected to administer only a single dose (1 mg/kg) of the MAb to the two vaccinated animals. Blood was also collected from monkey BF17, which had been immunized but not treated with anti-CD8 MAb, and monitored in parallel for levels of CD8+ T cells and for viral RNA loads in plasma. As shown in Fig. 6A, circulating CD8+ T lymphocytes became undetectable within 24 h of anti-CD8 MAb administration; no concomitant loss of CD4+ T or CD20+ B cells was observed (not shown). The loss of CD8+ T cells was accompanied by a rapid increase of viral RNA in the plasma of both treated monkeys. In the case of macaque BF08, this represented a 50-fold rise to 2.5 × 10^9 viral RNA copies/ml from the pretreatment level of 490 copies/ml (Fig. 6B). The increased viral loads were short-lived for both animals, as the viral loads returned to pretreatment levels over a 9- to 11-day period which coincided with the recovery of CD8+ T cells to near predepletion values. These results indicate that CD8-mediated cellular responses contributed to the suppression of virus replication in the vaccinated animals.

Long-term follow-up of vaccinated monkeys. At the time this report was written, the four vaccinated animals were still alive >60 weeks post-virus challenge. As shown in Fig. 7, all had fluctuating and detectable plasma viremia in the vicinity of 10^5 RNA copies/ml of plasma. Their CD4+ T-cell counts were at prechallenge levels, and all four monkeys remained free of clinical symptoms.

**DISCUSSION**

The main purpose of this study was to assess the use of inactivated virus particles to boost immune responses initially

| Animal | Neutralizing Ab response at indicated wk postchallenge | Anti-SHIVDH12R-PS1 | Anti-week 22 virusb |
|--------|--------------------------------------------------------|---------------------|-------------------|
|        | 0        | 4        | 20       | 0        | 4        | 20       |
| BF08   | +        | +        | +        | −        | −        | −        |
| BF16   | +        | +        | +        | −        | −        | −        |
| BF17   | +        | +        | +        | +        | −        | −        |
| BF35   | +        | +        | +        | −        | −        | −        |

*All plasma was tested at a 1:10 dilution. +, presence of Ab; −, absence of Ab.

b Virus was isolated from all four vaccinated monkeys at week 22, and neutralization sensitivity to plasma samples collected at week 20 was assessed.
primed by rVVsvs. Because inactivation with AT-2 preserves the functional properties of envelope glycoproteins present on HIV-1 virions (66), it was thought that boosting with preparations containing trimeric gp120/gp41 molecules in their native conformation might elicit broadly reactive neutralizing Abs, capable of suppressing infections initiated by viruses containing heterologous envelopes. Furthermore, since inactivated SIV and HIV-1 particles also contain the full complement of virion-associated proteins, this vaccine regimen had the potential for presenting additional viral antigens to both arms of the immune system. This immunization approach did, in fact, elicit levels of neutralizing Abs recently shown to confer >99% sterilizing protection to rhesus monkeys exposed to 75 TCID50 of SHIVDH12 (53). Unfortunately, the neutralizing activity was only type specific and failed to block the closely related and highly pathogenic SHIVDH12R-PS1 used for challenge. This inability of virion-associated gp120 to broaden the neutralizing Ab response is reminiscent of the reported failure of monkeys chronically infected with other SHIVs to generate cross-protective Abs against SHIVs bearing a heterologous envelope glycoprotein (27). It is possible that the lack of breadth observed in our study might be related to the small amount of virion-associated HIV-1DH12 envelope administered during the AT2 virion immunizations (0.5 g of total gp120 per animal [E. Chertova, personal communication]) and the level of type-specific responses observed (Table 1). We are currently making progress towards generating inactivated HIV and SHIV preparations of greater purity and with higher envelope content (J. Lifson, unpublished data). The planned use of oligomeric HIV-1 Env immunogens to broaden humoral immune responses (4, 12, 23, 28, 83) may also require additional structural modifications such as variable loop deletions (4, 17, 76, 81, 82), removal of glycosylation sites (63), or novel alterations that will expose potential conserved epitopes.

The four vaccinated animals used in the present study were protected against the rapid depletion of CD4+ T cells and controlled postpeak viremia after challenge with the highly pathogenic SHIVDH12R-PS1 to low but detectable levels. This result is similar to those of several recently reported prime and boost studies in which immunized monkeys were challenged with a different highly pathogenic SHIV, SHIV89.6P (2, 7, 8, 18, 65, 74). In several of those experiments, potent antiviral cellular immune responses were measured both prior to and following SHIV89.6P inoculation. At present we have no explanation for the inability to detect cellular immune responses directed against SHIVDH12R-PS1 prior to challenge. The rapid increases of viral RNA levels in plasma observed following the anti-CD8 MAb-induced depletion of CD8+ T cells in two vaccinated animals 27 weeks after challenge indicates that this T-lymphocyte subset was playing a role in controlling virus replication. We also measured high titers of anti-SHIVDH12R-PS1 neutralizing Abs by 3 weeks postinfection in all four immunized monkeys (Table 2), suggesting that this early humoral response may have helped to suppress virus replication and prevent the onset of disease. In this regard, we previously reported that macaques inoculated with very low amounts of infectious virus usually experience minimal or partial depletions of CD4+ T lymphocytes and are able to generate neutralizing Abs against SHIVDH12R (26). However, the neutralizing activity in several of these infected animals became detectable only between 10 and 20 weeks postinfection. Since infection of naive monkeys with high multiplicities of infection of SHIVDH12R is invariably associated with rapid and irreversible depletions of CD4+ T cells and no demonstrable antiviral humoral responses (26), preservation of this lymphocyte subset in the vaccinated animals contributed to a rapid and vigorous neutralizing Ab response.

The existence of both cellular and humoral antiviral responses in the vaccinated monkeys after challenge suggests...
that both arms of the immune system were participating in suppressing virus replication and preventing disease, although a direct correlation could not be conclusively established. In fact, the contribution of the humoral response observed may be questionable in view of the recovery of replication-competent virus from monkey BF17, which remained susceptible to contemporaneously circulating neutralizing Ab. The recovery of replication-competent virus from all four vaccinated animals (at 22 weeks postchallenge) indicates that immunologic control, in general, was still incomplete, permitting the establishment of persistent infections. Evidence supporting this conclusion was that although all four monkeys continued to generate neutralizing Abs against the input SHIV\textsubscript{D112R-PS1} throughout the postchallenge period, virus isolated from three of these animals at week 22 postinfection was refractory to neutralization when plasma samples collected at weeks 4 and 20 postchallenge were used. In addition, the virus recovered from one of the vaccinated animals at week 22 postinfection promptly induced the prototypic rapid and irreversible loss of CD4\textsuperscript{+} T cells following its inoculation into a naive macaque. The fact that neutralization escape variants evolved in three of the monkeys is indicative that the Ab response is exerting substantial selective pressure on the virus population. Further monitoring of our vaccinated animals will be needed to determine whether eventual loss of immunologic control and disease progression occurs, as has been recently reported for a rhesus monkey vaccinated against SHIV\textsubscript{89.6P} (6).

Although our results and similar findings by others using prime and boost regimens to control the postpeak viremia attending virus challenges with highly pathogenic SHIVs appear to be encouraging advancements in the quest to develop an effective vaccine against HIV-1, comparable vaccine approaches, when applied to the SIV animal model system, only transiently control virus load (1, 10, 33, 45, 55, 60). In most approaches, when applied to the SIV animal model system, only effective vaccine against HIV-1, comparable vaccine approaches when attending virus challenges with highly pathogenic SHIVs undoubtedly reflect major differences in their respective intrinsic biologic properties. Even though both viruses achieve comparable peak virus loads (10\textsuperscript{7} to 10\textsuperscript{8} viral RNA copies/ml of plasma) during primary infection, a variety of interventions (viz., vaccination, short-term antiretroviral therapy, and passive immunotherapy) or the conditions of in vivo infection (low virus inoculum size) can readily suppress the SHIV RNA, but not SIV RNA, in plasma to very low levels for extended periods of time (2, 7, 8, 18, 26, 36, 46, 53, 58, 65, 74; R. Willey, Y Endo, and M. Martin, unpublished data). This durable control of pathogenic SIV infections in vivo may be a consequence of rapidly and effectively suppressing the systemic dissemination of virus during the initial weeks of infection. Thus, in contrast to SIV (and possibly HIV-1 also), SHIVs may only rarely escape from imposed early-phase restrictions and are, in general, unable to regain sufficient replicative vigor to overcome immunologic control.

Following resolution of the primary infection, the viral RNA levels in the plasma of our four immunized monkeys over a 60-week observation period (Fig. 7) appear to be somewhat higher than the set point values reported in similar vaccine experiments utilizing SHIV\textsubscript{89.6P} (2, 7, 8, 65). This could reflect our use of a less effective vaccination protocol or suggests that SHIV\textsubscript{D112R-PS1} possesses more aggressive in vivo replicative properties. The latter is consistent with several reports showing that SHIV\textsubscript{89.6P} inoculation induces minimal or partial depletions of CD4\textsuperscript{+} T lymphocytes in some naive or vaccinated control animals (8, 65, 74, 75). In addition, we have conducted PCR analyses of both virus stocks and have found that 12 of 12 env gene segments from SHIV\textsubscript{89.6P} contained a truncated HIV-1 gp41 that was joined, in frame, to SIV gp41/Nef-coding sequences, as has previously been reported (40). In contrast, a similar analysis of SHIV\textsubscript{D112R-PS1} revealed an intact HIV-1 gp41. Taken together, these results indicate that SHIV\textsubscript{D112R-PS1} differs biologically and genetically from SHIV\textsubscript{89.6P} and may be able to mount more-vigorous and unremitting infections of rhesus monkeys.

The perplexing problem currently facing the HIV vaccine field is whether containment of an established persistent infection will be sufficient to prevent subsequent immunodeficiency. It is widely recognized that most effective prophylactic vaccines directed against viral pathogens prevent disease, not infection. Although several immunologic and virologic markers have been associated with progression to disease, the viral set point is currently thought to be the best parameter for prognosticating the HIV-1 clinical course (49). One could argue, however, whether a vaccine strategy designed to control postpeak viremia is applicable to a family of viruses that readily establish persistent infections and generate progeny virions which frequently escape immunologic surveillance and/or antiretroviral therapy. Furthermore, none of the vaccine regimens used in SIV and SHIV studies have reduced the initial peak viremia to <10\textsuperscript{6} RNA copies/ml of plasma. More-stringent suppression of this burst of virus production may be required to curtail the systemic dissemination of progeny virions, reduce the integration of proviral DNA into long-lived reservoirs such as memory T cells and tissue macrophage, and minimize the emergence of viral variants capable of escaping immunologic control.

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REFERENCES

1. Allen, T. M., L. Mortara, B. R. Mothe, M. Lieb, P. Jing, B. Calore, M. Piekarzcyk, R. Rueddersdorf, D. H. O’Connor, X. Wang, C. Wang, D. B. Allison, J. D. Altman, A. Sette, R. C. Desrosiers, G. Sutter, and D. L. Watkins. 2002. Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. J. Virol. 76:4108–4112.

2. Amara, R. R., F. Villing, J. D. Altman, S. D. Lydy, S. P. O’Neill, S. I. Staaprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A. Candido, N. L. Kooey, P. L. Earl, J. M. Smith, H.-L. Ma, B. D. Grimm, M. L. Hulsey, J. Miller, H. M. McClure, J. M. McNicholl, B. Moss, and H. L. Robinson. 2001. Control of a mucosal challenge and prevention of AIDS by a multipeptide DNA/MVA vaccine. Science 292:69–74.

3. Arthur, L. A., J. W. Bess, Jr., E. N. Chertovs, J. L. Rossio, M. T. Esser, R. E. Benveniste, L. E. Henderson, and J. D. Lifson. 1998. Chemical inactivation...
of retroviral infectivity by targeting nucleocapsid protein zinc fingers: a candidate SIV vaccine, AIDS Res. Hum. Retrovir. 14(Suppl. 3):S311–S319.

10. Cho, M. W., Y. B. Kim, M. K. Lee, K. C. Gupta, D. M. Irbeck, A. G. Gaitan, T. Zhu, C. Graziosi, K. M. McGrath, J. Tartaglia, P. Buge, S. L., E. Richardson, S. Alipanah, P. Markham, S. Cheng, N. Kalyan, Binley, J. M., R. W. Sanders, B. Clas, N. Schuelke, A. Master, Y. Guo, F. Dubey, D. Casimiro, A. Simon, M.-E. Davies, M. Chastain, T. B. Strom, R. S. Lifton, C. E. Nickerson, W. I. Cox, J. McNamara, K.-L. Hwang, Excler, A.-M. Duliege, J. Tartaglia, W. I. Cox, J. McNamara, K.-L. Hwang, against the highly pathogenic simian immunodeficiency virus type 1 (HIV-1) envelope antigenic mimic of the trimeric virion-associated glycoprotein. J. Virol. 72:2155–2166.

11. Davis, N. L., L. J. Caley, K. W. Brown, M. R. Betts, D. M. Irbeck, K. M. McGrath, M. J. Connell, D. C. Montefiori, D. A. Frelinger, R. Swanson, P. R. Johnson, and R. E. Johnston. 2000. Vaccination of macaques against pathogenic simian immunodeficiency virus with Venezuelan equine encephalitis virus replicon particles. J. Virol. 74:371–378.

12. Earl, P. L., C. C. Broder, D. Long, S. A. Lee, J. Peter, S. Chakrabarti, R. W. Doms, and B. Moss. 1994. Native oligomeric human immunodeficiency virus envelope glycoprotein elicits diverse monoclonal antibody reactivities. J. Virol. 68:3015–3026.

13. Egkan, M. A., W. A. Pavlat, J. Tartaglia, E. Paoletti, K. J. Weinhold, M. L. Clements, and R. F. Siliciano. 1995. Induction of human immunodeficiency virus type 1 (HIV-1)-specific cytolytic T lymphocyte responses in seronegative rhesus monkeys by a nonreplicating, host-range-restricted canarypox vector (ALVAC) carrying the HIV-1 envelope gene. J. Infect. Dis. 171:823–827.

14. Emeri, E. A., W. A. Schiefl, J. H. Nix, J. A. Conley, E. A. Ef, J. H. Nunberg, A. J. Conley, Y. Eda, T. Yoshio, S. D. Putney, S. Matsuhashi, E. K. Cobb, C. M. Jett, et al. 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature 355:728–730.

15. Endo, Y., T. Igarashi, Y. Nishimura, C. Buckler, A. Buckler-White, R. Plishka, D. S. Dimitrov, and M. A. Martin. 2000. Short- and long-term clinical outcomes in rhesus monkeys inoculated with a highly pathogenic chimeric simian/human immunodeficiency virus. J. Virol. 74:6935–6945.

16. Etemad-Moghaddam, B., G. B. Karlsson, M. Halloran, Y. Sun, D. Schenten, M. Fernandes, N. L. Letvin, and J. Sodroski. 1998. Characterization of simian-human immunodeficiency virus envelope glycoprotein epitopes recognized by neutralizing antibodies in infected monkeys. J. Virol. 72:4387–4345.

17. Farzan, M., H. Choe, E. Desjardins, Y. Sun, K. Juhn, C. A. Danchamh, B. Kolchinsky, M. Koch, R. Wyatt, and J. Sodroski. 1998. Stabilization of human immunodeficiency virus type 1 envelope glycoprotein trimers by disulfide bonds introduced into the gp41 envelope ectodomain. J. Virol. 72:7620–7625.

18. Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss. 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA 83:8122–8126.

19. Gauduin, M. C., P. W. Parren, R. Weir, C. F. Barbosa, D. R. Burton, and R. A. Koup. 1997. Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat. Med. 3:1389–1393.

20. Graham, B. S., M. J. McElrath, R. I. Connor, D. H. Schwartz, G. J. Gore, M. C. Keffer, M. J. Mulligan, T. J. Matthews, S. M. Wolinsky, D. C. Montefiori, S. H. Vermund, R. A. Lambart, L. Corey, R. B. Belshe, R. Dolin, P. F. Wright, B. T. Kotze, M. C. Wolf, P. E. Fast, the AIDS Vaccine Evaluation Group, and the Correlates of HIV Immune Protection Group. 1998. Analysis of intercurrent human immunodeficiency virus type 1 infections in phase I and II trials of candidate AIDS vaccines. J. Infect. Dis. 177:310–319.

21. Hanke, T., T. J. Blanchard, J. Schneider, C. M. Hannan, M. Becker, S. C. Gilbert, A. V. Hill, G. L. Smith, and A. Michael. 1998. Enhancement of MHC class I-restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination regimen. Vaccine 16:393–445.

22. Hanke, T., R. V. Samuel, T. J. Blanchard, V. C. Neumann, T. M. Allen, J. E. Boyson, S. A. Sharpe, N. Cook, G. L. Smith, D. I. Watkins, M. P. Cranage, and A. J. Michael. 1999. Effective induction of simian immunodeficiency virus-specific cytolytic T lymphocytes in macaques by using a multipeptide gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. J. Virol. 73:7528–7536.

23. Harada, S., Y. Koyanagi, and N. Yamamoto. 1985. Infection of HTC-III/LAV in HTLV-I-carriers cells MT-2 and MT-4 and application in a plaque assay. Science 229:563–566.

24. Hasenkugl, K. J., and U. Dittmer. 2000. The role of CD4 and CD8 T cells in recovery and protection after homologous or heterologous HIV-1 primary infection: lessons from the Friend virus model. Virology 272:244–249.

25. Igarashi, T., C. R. Brown, Y. Endo, A. Buckler-White, R. Plishka, N. Bischofberger, V. Hirsch, and M. A. Martin. 2001. Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV
type 1 chimera (SHIV): implications for HIV-1 infections of humans. Proc. Natl. Acad. Sci. USA 98:658–663.

37. Metzner, K. J., Y. Nishimura, R. J. Donahoe, D. Radlinski, R. Sajjadianpur, T. Matano, C. Bucker, A. Bucker-White, R. Pfister, T. Theodore, R. Shibata, and M. Martin. 1999. Emergence of a highly pathogenic simian/human immunodeficiency virus in a rhesus macaque treated with anti-CD8 mAb during a primary infection with a nonpathogenic simian immunodeficiency virus. Proc. Natl. Acad. Sci. USA 96:14043–14048.

38. Jin, X., D. E. Bauer, P. A. Settleton, S. Levin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrickis, L. Zhang, A. S. Perelson, and D. D. Ho. 1999. Dramatic rise in plasma viremia after CD8⁺ T cell depletion simian immunodeficiency virus-infected macaques. J. Exp. Med. 189:991–998.

39. Sonner, M., F. J. Nooj, P. van Suylenhjem, P. Neuhaus, and G. Goldstein. 1986. The influence of OKT8 treatment on allograft survival in rhesus monkeys. Transplantation 41:431–435.

40. Karlsson, G. B., M. Halloran, J. Li, I. W. Park, R. Gomila, K. A. Reimann, M. K. Axthelm, S. A. Hill, N. L. Letvin, and J. Sodroski. 1997. Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4⁺ lymphocyte depletion in rhesus monkeys. J. Virol. 71:4128–4225.

41. Kent, S. J., A. Zhao, S. J. Best, J. D. Chandler, D. R. Boyle, and I. A. Ramshaw. 1999. Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of concomitant priming with DNA and boosting with recombinant fowlpox virus. J. Virol. 72:10180–10188.

42. Kessler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Betzler, P. Sehgal, D. Daniel, N. King, et al. 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 248:1109–1112.

43. Safrit, T. J., T. C. VanCott, Y. Cao, C. A. Andrews, G. McLeod, W. Borkovsky, C. Farching, and D. D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. 68:4650–4655.

44. lifton, P. R., I. W. Park, R. Arnaout, L. L. T. Parks, D. K. Schneider, R. F. Kiser, V. Goehler, W. R. Alls, J. M. Fischer, R. B. M. Flynn, N. Bischoffer, M. Piatak, Jr., V. M. Hirsch, M. A. Nowak, and D. Wodarczak. 2001. Containment of simian immunodeficiency virus infection: cellular immune responses and protection from rechallenge following transient postocclusion antiretroviral treatment. J. Virol. 75:2584–2593.

45. Lu, S., J. Arthos, D. C. Montefiori, Y. Yasutomi, K. Manson, F. Mustafa, E. Johnson, J. C. Santoro, J. Wissink, J. L. Mullins, J. R. Haynes, N. L. Letvin, M. Wyand, and H. L. Robinson. 1998. Simian immunodeficiency virus DNA vaccine trial in macaques. J. Virol. 70:3978–3991.

46. Mascia, R. J., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. O’Lourer, C. R. Brown, C. W. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, T. S. A. Hu, M. L. H. O. Arthur, L. E. Henderson, and J. D. Lifson. 1996. The inutility of OKT8F treatment on allograft survival in rhesus monkeys. Transplantation 61:529–534.

47. Matano, T., R. Shiba, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin. 1998. Administration of an anti-CD100 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J. Virol. 72:164–169.

48. Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272:1167–1170.

49. Metzner, K. J., X. Jin, F. V. Lee, A. Gettie, D. E. Bauer, M. D. Mascio, A. Perelson, T. Marz, D. Ho, L. G. Kostrickis, and R. J. Connolly. 1996. Effects of in vivo CD8⁺ T cell depletion on virus replication in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. J. Exp. Med. 191:1921–1931.

50. Moore, J. P., Y. Cao, L. Qing, G. J. Sattentrant, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas III, D. R. Burton, and D. D. Ho. 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monoclonic gp120. J. Virol. 69:101–109.

51. Masure, L., J. Hughes, T. Schacker, T. Shea, L. Corey, and M. J. McElrath. 1997. Cytotoxic T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. N. Engl. J. Med. 337:1267–1272.

52. Nishimura, Y., T. Igarashi, N. Haigwood, R. Sajjadianpur, R. J. Plishka, A. Bucker-White, R. Shibata, and M. Martin. 2002. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J. Virol. 76:2123–2130.
R. Willey, M. W. Cho, and M. A. Martin. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-USIV chimeric virus infections of macaque monkeys. Nat. Med. 5:204–210.

72. Shibata, R., F. Maldarelli, C. Siemon, T. Matano, M. Parta, G. Miller, T. Fredrickson, and M. A. Martin. 1997. Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. J. Infect. Dis. 176:362–373.

73. Shibata, R., C. Siemon, M. W. Cho, L. O. Arthur, S. M. Nigida, Jr., T. Matthews, L. A. Sawyer, A. Schultz, K. K. Murthy, Z. Israel, A. Javadian, P. Frost, R. C. Kennedy, H. C. Lane, and M. A. Martin. 1996. Resistance of previously infected chimpanzees to successive challenges with a heterologous intraclade B strain of human immunodeficiency virus type 1. J. Virol. 70:4361–4369.

74. Shiver, J. W., T. M. Fu, L. Chen, D. R. Casimiro, M. E. Davies, R. K. Evans, Z. Q. Zhang, A. J. Simon, W. L. Trigona, S. A. Dubey, L. Huang, V. A. Harris, R. S. Long, X. Liang, L. Handt, W. A. Schleif, L. Zhu, D. C. Freed, N. V. Persaud, L. Guan, K. S. Pun, A. Tang, M. Chen, K. A. Wilson, K. B. Collins, G. J. Heidecker, V. R. Fernandez, H. C. Perry, J. G. Joyce, K. M. Grimm, J. C. Cook, P. M. Keller, D. S. Kresock, H. Mach, R. D. Troutman, L. A. Isopi, D. M. Williams, Z. Xu, K. E. Bohannon, D. B. Volkin, D. C. Montefiori, A. Miura, G. R. Krivulka, M. A. Lifton, M. J. Kuroda, J. E. Schmitz, N. L. Letvin, M. J. Caulfield, A. J. Beit, R. Youil, D. C. Kaslow, and M. A. Martin. 1998. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J. Virol. 62:139–147.

75. Wrin, T., and J. H. Nunberg. 1994. HIV-1MN recombinant gp120 vaccine serum, which fails to neutralize primary isolates of HIV-1, does not antagonize neutralization by antibodies from infected individuals. AIDS 8:1622–1623.

76. Willey, R. L., R. Shibata, E. O. Freed, M. W. Cho, and M. A. Martin. 1996. Differential glycosylation, virion incorporation, and sensitivity to neutralizing antibodies of human immunodeficiency virus type 1 envelope produced from infected primary T-lymphocyte and macrophage cultures. J. Virol. 70:6431–6436.

77. Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. J. Capon, and M. A. Martin. 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J. Virol. 62:139–147.

78. Wyatt, R., N. Sullivan, M. Thali, H. Regue, D. Ho, J. Robinson, M. Posner, and J. Dodroski. 1995. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. J. Virol. 69:5723–5733.

79. Wyatt, R., N. Sullivan, M. Thali, H. Regue, D. Ho, J. Robinson, M. Posner, and J. Dodroski. 1993. Functional and immunologic characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions. J. Virol. 67:4557–4565.

80. Yang, X., L. Florin, M. Farzan, P. Kolchinsky, P. D. Kwong, J. Sodroski, and R. Wyatt. 2000. Modifications that stabilize human immunodeficiency virus envelope glycoprotein trimers in solution. J. Virol. 74:4746–4754.