JOURNAL OF VIROLOGY, Jan. 2003, p. 1163–1174 Vol. 77, No. 2
0022-538X/03/$08.00+0 DOI: 10.1128/JVI.77.2.1163–1174.2003

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

Ronald L. Willey,[1] Russ Byrum,[2] Michael Piatak,[3] Young B. Kim,[1] Michael W. Cho,[1] Jeffrey L. Rossio, Jr.,[3] Julian Bess, Jr.,[3] Tatsuhiko Igarashi,[1] Yasuyuki Endo,[1] Larry O. Arthur,[3] Jeffrey D. Lifson,[3] and Malcolm A. Martin[1]*

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892[1]; Bioqual, Rockville, Maryland 20850[2]; and AIDS Vaccine Program, SAIC Frederick, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702[3]

Received 31 July 2002/Accepted 7 October 2002

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 SIV_{mac} 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

* Corresponding author. Mailing address: Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 4, Rm. 315, 4 Center Dr., MSC 0460, Bethesda, MD 20892. Phone: (301) 496-4012. Fax: (301) 402-0226. E-mail: malm@nih.gov.

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 with the R5-utilizing $SHIV_{162P4}$ in a dose-dependent fashion (58). Similarly, polyclonal neutralizing immunoglobulin G (IgG), derived from chimpanzees chronically infected with HIV-1_{DH12} (73), can protect 99% of pigtailed macaques from an intravenous exposure to 75 50% tissue culture infective doses ($TCID_{50}$) of $SHIV_{DH12}$ if a neutralization titer of 1:38 in plasma is achieved (53). Unfortunately, no currently available viral envelope immunogen is able to elicit this level of 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 $SHIV_{DH12R}$-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. Aldrithiol-2 (AT-2)-inactivated particles were administered to boost immune responses initially primed with a mixture of recombinant vaccinia viruses (rVVs) 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) $SHIV_{DH12R-PS1}$, 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 naïve 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 24 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 $SHIV_{DH12}$ has been previously reported (72). The highly pathogenic $SHIV_{DH12R}$ 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 $SHIV_{DH12}$ (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 $SHIV_{DH12R-PS1}$ (Fig. 1). A tissue culture stock of $SHIV_{DH12R-PS1}$ 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 naïve rhesus monkey. The titers of both $SHIV_{DH12}$ (used for immunoblotting and virus neutralization assays) and $SHIV_{DH12R-PS1}$ (used as the challenge virus and in neutralization assays) were measured by end point dilution in rhesus PBMC and MT-4 cells. The $TCID_{50}$ for each virus stock was calculated by the method of Reed and Muench (61).

rVVs and inactivated SIV and HIV-1 virions. The construction and use of rVVs that express the HIV-1_{DH12} gp160 envelope (vvDHenv) and the T7 RNA polymerase (vTF7-3) have been previously described (19, 29). An additional rVV which expresses the SIV_{mac239} *gag* and *pol* polyproteins (vvSIVgag-pol) was generated; the details of the construction will be described elsewhere (Y. B. Kim and M. W. Cho, unpublished data).

HIV-1_{DH12} (70) and SIV_{mac239} (42) particles in the supernatants of large-scale cultures of infected CEM X174(T1) cells were inactivated with 1 mM AT-2 as described previously (3). AT-2-treated particles were recovered by continuous-flow ultracentrifugation and concentrated by pelleting in an ultracentrifuge. 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.

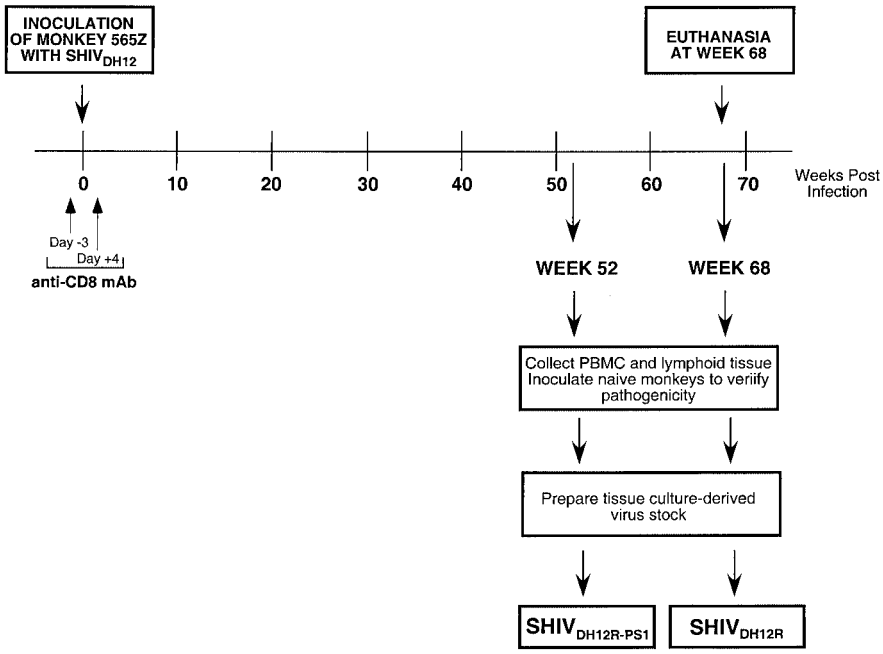


FIG. 1. Origin of the pathogenic SHIVs used in this study.

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 vvSIVgag-pol (5×10^7 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 μg of p24) and SIV$_{mac239}$ (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 (10^8 PFU) followed by intramuscular injections of AT-2-treated microvesicle preparations (2.5 mg of protein) in combination with QS21.

Detection of humoral responses. Plasma was prepared from ACD-treated whole-blood samples collected throughout the pre- and postchallenge phases of the study. Selected samples were screened for the presence of SIV- and HIV-1-specific Ab responses by immunoblot analysis as previously described (37). Briefly, lysates from concentrated SHIV$_{DH12}$ virions were supplemented with 300 ng of purified recombinant HIV-1$_{DH12}$ gp120H (19), resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to Immobilon-P (Millipore Corporation, Bedford, Mass.) membranes. The membranes were then incubated with 1:100 dilutions of plasma that had been preabsorbed with lysates from *Escherichia coli* and MT-4 cells and then incubated with goat anti-human IgG-conjugated horseradish peroxidase (Amersham, Piscataway, N.J.). Protein bands were visualized on X-ray film following incubation with a chemiluminescent substrate (SuperSignal; Pierce, Rockford, Ill.).

Virus neutralization assay. Plasma samples were screened for neutralizing activity by an assay that measures 100% virus neutralization (73, 78). Selected samples were treated at 56°C for 30 min, insoluble material was removed by pelleting in an Eppendorf centrifuge, and the remaining supernatant was filtered through a 0.45-μm-pore-size Quick Spin filter (Corning Lifesciences, Acton, Mass.) prior to use. A total of 100 TCID$_{50}$ of SHIV$_{DH12}$ or SHIV$_{DH12R-PS1}$ in 25 μl of RPM–FBS–IL-2 medium was mixed with 25 μl of medium containing a 1:5 dilution of each plasma sample (final concentration in plasma, 1:10) in quadruplicate 96-flat-bottom-well plates. After incubation at 37°C for 1 h, 5×10^4 freshly prepared and mitogen-activated rhesus PBMC in 150 μl of RPMI–FBS–IL-2 medium were added to each well and the cultures were maintained at 37°C in 5% CO_2. On day 6, cells were diluted 1:2 with 100 μl of medium containing 5×10^4 freshly prepared and activated rhesus PBMC and incubated for an 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.

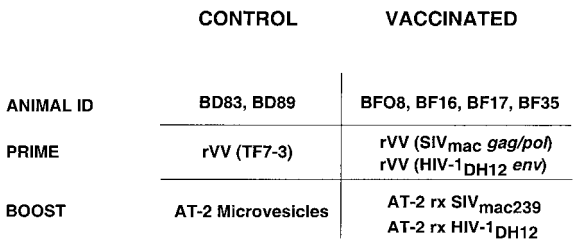
Animal challenge, virus loads, and in vivo lymphocyte analyses. All animals were challenged intravenously with 500 TCID$_{50}$ (based on the titer in MT4 cells) of the highly pathogenic SHIV$_{DH12R-PS1}$ 18 weeks following the final immunization. End point titrations of the SHIV$_{DH12R-PS1}$ challenge 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 (4.0×10^6) were cocultured with freshly prepared and mitogen-activated PBMC (1.5×10^6) 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 (10^4 TCID$_{50}$) 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 OKT8F (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 MAb/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.



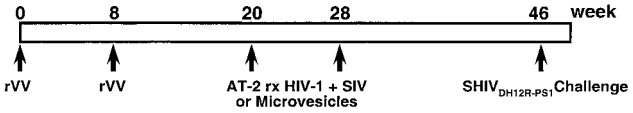


FIG. 2. Vaccine study groups, immunogens, and schedule. Viral antigen-vaccinated animals ($n = 4$) were immunized twice (weeks 0 and 8) with rVVs expressing the SIV Gag/Pol and HIV-1 Env proteins followed by intramuscular injections with AT-2-inactivated SIV and HIV-1 virions (weeks 20 and 28). Two control animals received rVV expressing the T7 polymerase followed by AT-2-treated microvesicles at the same time intervals. All animals were challenged with $SHIV_{DH12R-PS1}$ 18 weeks following the last immunization.

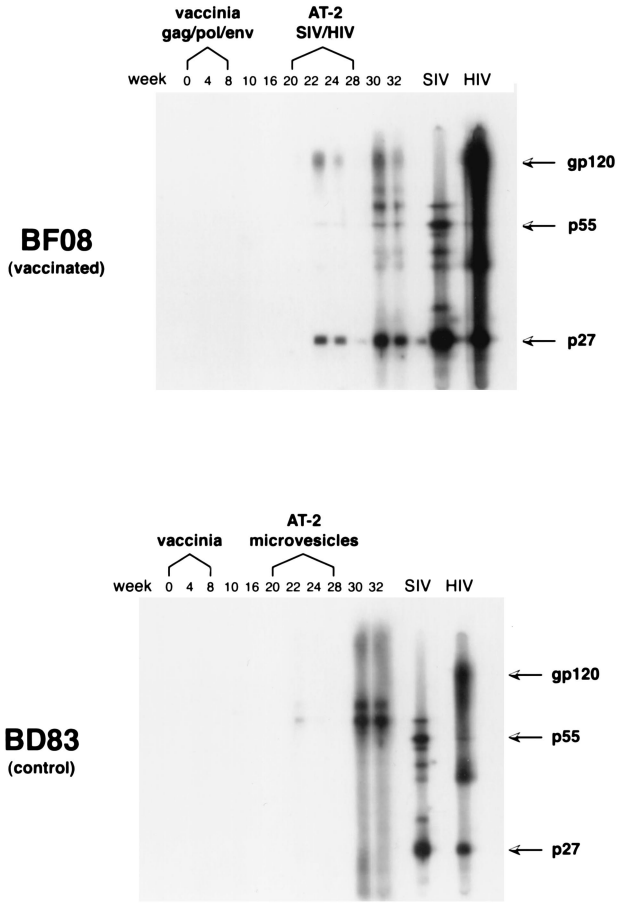


FIG. 3. Immunoblot reactivity to SIV and HIV-1 proteins in plasma samples collected from representative vaccinated (BF08) (upper panel) and control (BD83) (lower panel) animals. The times of immunization and samples analyzed are indicated. Lanes designated SIV and HIV show reactivity profiles of control sera collected from SIV-infected monkeys and HIV-infected humans; the positions of the gp120 Env, p55, and p27 Gag proteins are indicated.

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 a mixture of two rVVs (5×10^7 PFU of each) expressing SIV_{mac239} Gag and Pol and the $HIV\text{-}1_{DH12}$ gp160 proteins. These four macaques subsequently received a booster with a mixture of AT-2-treated $HIV\text{-}1_{DH12}$ and SIV_{mac239} particles, in combination with QS21 adjuvant, at weeks 20 and 28. Two control monkeys were immunized in parallel with rVV vTF7-3, which expresses T7 RNA polymerase, and then with AT-2-treated cell-derived microvesicles.

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 $SHIV_{DH12}$ particles, to which $HIV\text{-}1_{DH12}$ 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 microvesicle 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 $SHIV_{DH12}$ (73, 78). Neutralizing activity against $SHIV_{DH12}$, 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 $TCID_{50}$ of $SHIV_{DH12}$, also prepared in rhesus monkey

TABLE 1. SIV- and/or HIV-1-specific immunoblot responses elicited prechallenge

Animal	Ab response after immunization with:			
	rVV		AT-2-treated microvesicles or virions[a]	
	Post-round 1	Post-round 2	Post-round 1	Post-round 2
Control macaques				
BD83	ND[b]	ND	ND	ND
BD89	ND	ND	ND	ND
Vaccinated macaques				
BF08	ND	ND	Gag/Env	Gag/Env
BF16	ND	ND	ND	Gag
BF17	ND	ND	Env	Gag/Env
BF35	ND	Env	Gag/Env	Gag/Env

[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 $SHIV_{DH12}$ (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 $SHIV_{DH12}$ 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-$SHIV_{DH12}$ 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 $SHIV_{DH12R-PS1}$, an uncloned highly pathogenic derivative of $SHIV_{DH12}$, which bears a gp120 containing multiple amino acid substitutions compared to the gp120 of $SHIV_{DH12}$, (37), was to be used as challenge virus in these animals, its sensitivity to neutralization was also determined. No anti-$SHIV_{DH12R-PS1}$ 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, $SHIV_{DH12R-PS1}$, neutralization resistant. This result is consistent with our previously reported failure to measure neutralizing Abs against $SHIV_{DH12R}$ in monkeys chronically infected with the closely related $SHIV_{DH12}$ (37; T. Igarashi, unpublished data) or to detect anti-$SHIV_{DH12R}$ neutralizing activity in IgG prepared from chimpanzees persistently infected with HIV-1_{DH12} (T. Igarashi, Y. Nishimura, and M. A. Martin, unpublished data).

TABLE 2. Anti-$SHIV_{DH12}$ neutralizing-AB titers elicited prechallenge

Animal	Neutralizing Ab titer determined after immunization with[a]:			
	rVV		AT-2-treated microvesicles or virions[b]	
	Post-round 1	Post-round 2	Post-round 1	Post-round 2
Control macaques				
BD83	ND	ND	ND	ND
BD89	ND	ND	ND	ND
Vaccinated macaques				
BF08	ND	ND	1:10	1:40
BF16	ND	ND	ND	ND
BF17	ND	ND	1:10	1:113
BF35	ND	1:10	1:10	1:63

[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 neutralizating-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 $SHIV_{DH12R-PS1}$. Eighteen weeks after the final immunization with the mixture of SIV and HIV-1 particles, all six animals were intravenously inoculated with 500 $TCID_{50}$ of the highly pathogenic $SHIV_{DH12R-PS1}$. The two control macaques, BD83 and BD89, developed peak virus loads of 2×10^8 and 7×10^7 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 $SHIV_{DH12R}$ 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.

$SHIV_{DH12R-PS1}$ 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 1.8×10^6 and 2.8×10^6 viral RNA copies/ml at weeks 2 to 3 postinoculation, while for the fourth macaque (BF17) the viremia was somewhat higher (1.6×10^7 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.

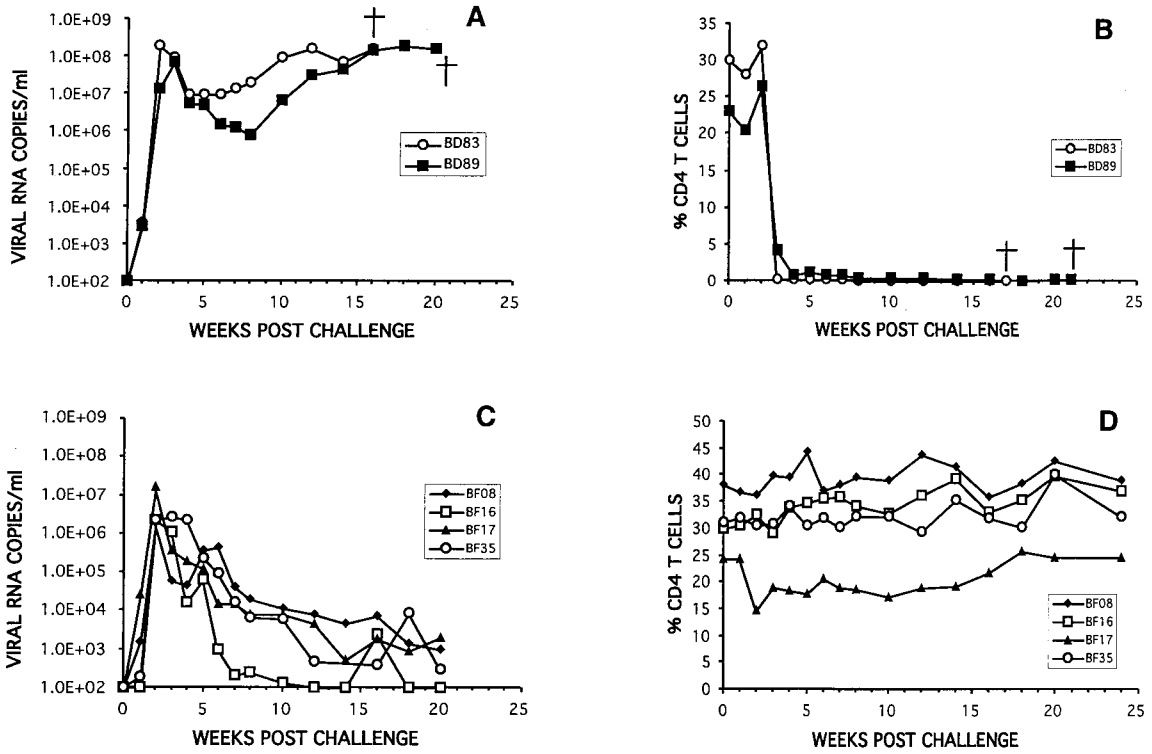


FIG. 4. Viral loads and $CD4^+$ T-cell levels in control and vaccinated animals following virus challenge. Animals were challenged with $SHIV_{DH12R-PS1}$ by intravenous inoculation. Blood collected from both groups of monkeys was screened for viral RNA (panels A and C) by RT-PCR, and the percentages of $CD4^+$ T cells in the peripheral blood (panels B and D) were determined by fluorescence-activated cell sorter analysis of lymphocyte subsets.

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.

TABLE 3. Anti-$SHIV_{DH12R-PS1}$ neutralizing Ab titers following virus challenge

Animal	Titer[a] of neutralizing Ab at wk:			
	1	2	3	12
Control macaques				
BD83	ND	ND	ND	ND
BD89	ND	ND	ND	ND
Vaccinated macaques				
BF08	ND	ND	1:66	1:63
BF16	ND	ND	1:1015	1:272
BF17	ND	ND	1:101	>1:320[b]
BF35	ND	ND	1:90	1:320

[a] Titer calculated by the method of Reed and Muench (60). ND, none detected at a 1:10 dilution.
[b] Virus neutralization end point not determined.

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

TABLE 4. Neutralizing Ab responses[a] against the input $SHIV_{DH12R-PS1}$ or autologous virus isolated from each vaccinated monkey at week 22 postchallenge

Animal	Neutralizing Ab response at indicated wk postchallenge					
	Anti-$SHIV_{DH12R-PS1}$			Anti-week 22 virus[b]		
	0	4	20	0	4	20
BF08	−	+	+	−	−	−
BF16	−	+	+	−	−	−
BF17	−	+	+	−	+	+
BF35	−	+	+	−	−	−

[a] 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.

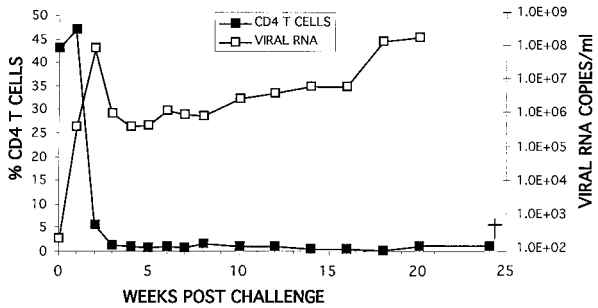


FIG. 5. In vivo properties of the virus recovered from vaccinated monkey BF16. The SHIV isolated from the PBMC of animal BF16 22 weeks after challenge was inoculated intravenously into a naïve rhesus macaque, VVF. The viral RNA loads and the percentages of $CD4^+$ T cells in the peripheral blood were determined as described in the legend to Fig. 4.

pathogenic properties compared to the starting inoculum. Virus was therefore isolated from all of the vaccinated animals at week 22 postchallenge by cocultivating their PBMC with PBMC from naïve rhesus monkeys. Neutralization assays were performed by using the $SHIV_{DH12R-PS1}$ challenge virus or the SHIV isolated at week 22 from each of the immunized animals and a 1:10 dilution of matched plasma samples collected from each monkey at weeks 0, 4, and 20 postchallenge. As expected from the results presented in Table 3, the plasma from week 4 readily neutralized the input $SHIV_{DH12R-PS1}$ (Table 4). The plasma collected on week 20 exhibited similar neutralizing activity against $SHIV_{DH12R-PS1}$, indicating the continued production of this neutralizing activity. In contrast, the SHIVs isolated at week 22 from monkeys BF08, BF16, and BF35 were refractory to neutralization when their matched plasma samples from weeks 4 and 20 were used. This result suggested that by week 22 postchallenge, the input $SHIV_{DH12R-PS1}$ had evolved to escape neutralization in these three animals. The virus recovered from macaque BF17 at week 22, on the other hand, remained sensitive to autologous Abs during the postchallenge period. Thus, in three of the four vaccinated animals, the persistently replicating SHIV had undergone sufficient antigenic change(s) to escape neutralization by Abs still capable of suppressing the input $SHIV_{DH12R-PS1}$.

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 $SHIV_{DH12R-PS1}$ (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 euthanized at week 25 because of its deteriorating clinical condition. Thus, although vaccination had prevented the invariably fatal $SHIV_{DH12R-PS1}$-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 rVVs expressing SIV and/or HIV-1 proteins elicit 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 $SHIV_{DH12R-PS1}$. 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 2.5×10^4 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 10^3 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

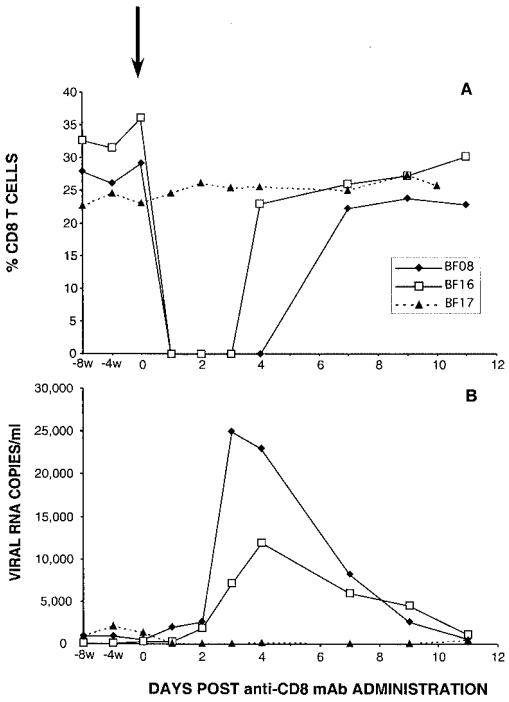


FIG. 6. In vivo $CD8^+$ T-cell depletions in vaccinated animals. The anti-human CD8 MAb OKT8F was administered intravenously (arrow) to macaques BF08 and BF16. The percentages of $CD8^+$ T cells in peripheral blood (A) were determined by fluorescence-activated cell sorter analysis, and viral RNA loads (B) were assessed by RT-PCR. The two points plotted before day 0 represent the levels of $CD8^+$ T cells and viral RNA 4 and 8 weeks prior to administration of the MAb. Untreated animal BF17 was monitored in parallel for comparison.

primed by rVVs. 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 $TCID_{50}$ of $SHIV_{DH12}$ (53). Unfortunately, the neutralizing activity was only type specific and failed to block the closely related and highly pathogenic $SHIV_{DH12R\text{-}PS1}$ 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 $HIV\text{-}1_{DH12}$ envelope administered during the AT2 virion immunizations (0.5 μg 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.

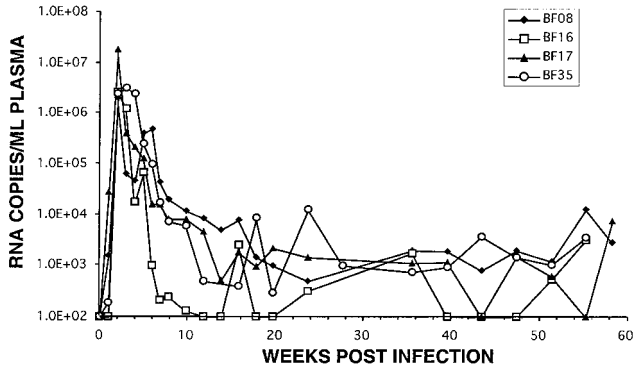


FIG. 7. Long-term virus replication profiles for the vaccinated animals.

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 $SHIV_{DH12R\text{-}PS1}$ 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, $SHIV_{89.6P}$ (2, 7, 8, 18, 65, 74). In several of those experiments, potent antiviral cellular immune responses were measured both prior to and following $SHIV_{89.6P}$ inoculation. At present we have no explanation for the inability to detect cellular immune responses directed against $SHIV_{DH12R\text{-}PS1}$ 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-$SHIV_{DH12R\text{-}PS1}$ 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 $SHIV_{DH12R}$ (26). However, the neutralizing activity in several of these infected animals became detectable only between 10 and 20 weeks postinfection. Since infection of naïve monkeys with high multiplicities of infection of $SHIV_{DH12R}$ 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 $SHIV_{DH12R-PS1}$ 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 $CD4^+$ T cells following its inoculation into a naïve 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 $SHIV_{89.6P}$ (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 instances, these immunization protocols have elicited robust cellular immune responses against SIV antigens yet were ineffective in preventing SIV-induced immunodeficiency in a majority of vaccinated animals. This has been the usual outcome even when the SIV challenge virus and the SIV proteins used for immunization were completely homologous. The discrepant results in protecting vaccinated macaques from pathogenic SIV versus SHIV infections undoubtedly reflect major differences in their respective intrinsic biologic properties. Even though both viruses achieve comparable peak virus loads (10^7 to 10^8 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 SHIV 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 $SHIV_{89.6P}$ (2, 7, 8, 65). This could reflect our use of a less effective vaccination protocol or suggests that $SHIV_{DH12R-PS1}$ possesses more aggressive in vivo replicative properties. The latter is consistent with several reports showing that $SHIV_{89.6P}$ inoculation induces minimal or partial depletions of $CD4^+$ T lymphocytes in some naïve 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 $SHIV_{89.6P}$ 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 $SHIV_{DH12R-PS1}$ revealed an intact HIV-1 gp41. Taken together, these results indicate that $SHIV_{DH12R-PS1}$ differs biologically and genetically from $SHIV_{89.6P}$ and may be able to mount more-vigorous and unrelenting 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 $<10^6$ 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.

ACKNOWLEDGMENTS

We thank Bernard Lafont for advice pertaining to the flow cytometry analyses, A. N. Hoffman and T. Parks for technical assistance with viral load determinations, A. Buckler-White and R. Plishka for the viral load assessments for animal VVF, and K. Demarest and T. Mercolino for kindly providing the anti-human CD8 monoclonal Ab OKT8F. We also thank S. Bour for help in preparing the figures.

This project has been funded in whole or in part with funds from the National Cancer Institute, National Institutes of Health, under contract no. NO1-CO-12400.

REFERENCES

1. **Allen, T. M., L. Mortara, B. R. Mothe, M. Liebl, P. Jing, B. Calore, M. Piekarczyk, R. Ruddersdorf, D. H. O'Connor, X. Wang, C. Wang, D. B. Allison, J. D. Altman, A. Sette, R. C. Desrosiers, G. Sutter, and D. I. Watkins.** 2002. Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. J. Virol. **76:**4108–4112.
2. **Amara, R. R., F. Villinger, J. D. Altman, S. L. Lydy, S. P. O'Neil, S. I. Staprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A. Candido, N. L. Kozyr, 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 multiprotein DNA/MVA vaccine. Science **292:**69–74.
3. **Arthur, L. O., J. W. Bess, Jr., E. N. Chertova, 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.
4. **Barnett, S. W., S. Lu, I. Srivastava, S. Cherpelis, A. Gettie, J. Blanchard, S. Wang, I. Mboudjeka, L. Leung, Y. Lian, A. Fong, C. Buckner, A. Ly, S. Hilt, J. Ulmer, C. T. Wild, J. R. Mascola, and L. Stamatatos.** 2001. The ability of an oligomeric human immunodeficiency virus type 1 (HIV-1) envelope antigen to elicit neutralizing antibodies against primary HIV-1 isolates is improved following partial deletion of the second hypervariable region. J. Virol. **75:**5526–5540.
5. **Barnett, S. W., S. Rajasekar, H. Legg, B. Doe, D. H. Fuller, J. R. Haynes, C. M. Walker, and K. S. Steimer.** 1997. Vaccination with HIV-1 gp120 DNA induces immune responses that are boosted by a recombinant gp120 protein subunit. Vaccine **15:**869–873.
6. **Barouch, D. H., J. Kunstman, M. J. Kuroda, J. E. Schmitz, S. Santra, F. W. Peyerl, G. R. Krivulka, K. Beaudry, M. A. Lifton, D. A. Gorgone, D. C. Montefiori, M. G. Lewis, S. M. Wolinsky, and N. L. Letvin.** 2002. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature **415:**335–339.
7. **Barouch, D. H., S. Santra, M. J. Kuroda, J. E. Schmitz, R. Plishka, A. Buckler-White, A. E. Gaitan, R. Zin, J.-H. Nam, L. S. Wyatt, M. A. Lifton, C. E. Nickerson, B. Moss, D. C. Montefiori, V. M. Hirsch, and N. L. Letvin.** 2001. Reduction of simian-human immunodeficiency virus 89.6P viremia in rhesus monkeys by recombinant modified vaccinia virus Ankara vaccination. J. Virol. **75:**5151–5158.
8. **Barouch, D. H., S. Santra, J. E. Schmitz, M. J. Kuroda, T.-M. Fu, W. Wagner, M. Bilska, A. Craiu, X. X. Zheng, G. R. Krivulka, K. Beaudry, M. A. Lifton, C. E. Nickerson, W. L. Trigona, K. Punt, D. C. Freed, L. Guan, S. Dubey, D. Casimiro, A. Simon, M.-E. Davies, M. Chastain, T. B. Strom, R. S. Gelman, D. C. Montefiori, M. G. Lewis, E. A. Emini, J. W. Shiver, and N. L. Letvin.** 2000. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science **290:**486–492.
9. **Belshe, R. B., G. J. Gorse, M. J. Mulligan, T. G. Evans, M. C. Keefer, J.-L. Excler, A.-M. Duliege, J. Tartaglia, W. I. Cox, J. McNamara, K.-L. Hwang, A. Bradney, D. Montefiori, and K. J. Weinhold for the NIAID AIDS Vaccine Evaluation Group.** 1998. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. AIDS **12:**2407–2415.
10. **Benson, J., C. Chougnet, M. Robert-Guroff, D. Montefiori, P. Markham, G. Shearer, R. C. Gallo, M. Cranage, E. Paoletti, K. Limbach, D. Venzon, J. Tartaglia, and G. Franchini.** 1998. Recombinant vaccine-induced protection against the highly pathogenic simian immunodeficiency virus SIV_{mac251}: dependence on route of challenge exposure. J. Virol. **72:**4170–4182.
11. **Bess, J. W., Jr., R. J. Gorelick, W. J. Bosche, L. E. Henderson, and L. O. Arthur.** 1997. Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. Virology **230:**134–144.
12. **Binley, J. M., R. W. Sanders, B. Clas, N. Schuelke, A. Master, Y. Guo, F. Kajumo, D. J. Anselma, P. J. Maddon, W. C. Olson, and J. P. Moore.** 2000. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J. Virol. **74:**627–643.
13. **Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone.** 1994. Virus-specific $CD8^+$ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. **68:**6103–6110.
14. **Buge, S. L., E. Richardson, S. Alipanah, P. Markham, S. Cheng, N. Kalyan, C. J. Miller, M. Lubeck, S. Udem, J. Eldridge, and M. Robert-Guroff.** 1997. An adenovirus-simian immunodeficiency virus *env* vaccine elicits humoral, cellular, and mucosal immune responses in rhesus macaques and decreases viral burden following vaginal challenge. J. Virol. **71:**8531–8541.
15. **Bures, R., A. Gaitan, T. Zhu, C. Graziosi, K. M. McGrath, J. Tartaglia, P. Caudrelier, R. El Habib, M. Klein, A. Lazzarin, D. M. Stablein, M. Deers, L. Corey, M. L. Greenberg, D. H. Schwartz, and D. C. Montefiori.** 2000. Immunization with recombinant canarypox vectors expressing membrane-anchored glycoprotein 120 followed by glycoprotein 160 boosting fails to generate antibodies that neutralize R5 primary isolates of human immunodeficiency virus type 1. AIDS Res. Hum. Retrovir. **16:**2019–2035.
16. **Burton, D. R., J. Pyati, R. Koduri, S. J. Sharp, G. B. Thornton, P. W. H. I. Parren, L. S. W. Sawyer, R. M. Hendry, N. Dunlop, P. L. Nara, et al.** 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science **266:**1024–1027.
17. **Cao, J., N. Sullivan, E. Desjardin, C. Parolin, J. Robinson, R. Wyatt, and J. Sodroski.** 1997. Replication and neutralization of human immunodeficiency virus type 1 lacking the V1 and V2 variable loops of the gp120 envelope glycoprotein. J. Virol. **71:**9808–9812.
18. **Chen, X., G. Scala, I. Quinto, W. Liu, T.-W. Chun, J. S. Justement, O. J. Cohen, T. C. vanCott, M. Iwanicki, M. G. Lewis, J. Greenhouse, T. Barry, D. Venzon, and A. S. Fauci.** 2001. Protection of rhesus macaques against disease progression from pathogenic SHIV-89.6PD by vaccination with phage-displayed HIV-1 epitopes. Nat. Med. **7:**1225–1231.
19. **Cho, M. W., Y. B. Kim, M. K. Lee, K. C. Gupta, W. Ross, R. Plishka, A. Buckler-White, T. Igarashi, T. Theodore, R. Byrum, C. Kemp, D. C. Montefiori, and M. A. Martin.** 2001. Polyvalent envelope glycoprotein vaccine elicits a broader neutralizing antibody response but is unable to provide sterilizing protection against heterologous simian/human immunodeficiency virus infection in pigtailed macaques. J. Virol. **75:**2224–2234.
20. **Connor, R. I., B. T. M. Korber, B. S. Graham, B. H. Hahn, D. D. Ho, B. D. Walker, A. U. Neumann, S. H. Vermund, J. Mestecky, S. Jackson, E. Fenamore, Y. Cao, F. Gao, S. Kalams, K. J. Kunstman, D. McDonald, N. McWilliams, A. Trkola, J. P. Moore, and S. M. Wolinsky.** 1998. Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines. J. Virol. **72:**1552–1576.
21. **Davis, N. L., I. J. Caley, K. W. Brown, M. R. Betts, D. M. Irlbeck, K. M. McGrath, M. J. Connell, D. C. Montefiori, J. A. Frelinger, R. Swanstrom, 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.
22. **Earl, P. L., C. C. Broder, D. Long, S. A. Lee, J. Peterson, S. Chakrabarti, R. W. Doms, and B. Moss.** 1994. Native oligomeric human immunodeficiency virus type 1 envelope glycoprotein elicits diverse monoclonal antibody reactivities. J. Virol. **68:**3015–3026.
23. **Earl, P. L., W. Sugiura, D. C. Montefiori, C. C. Broder, S. A. Lee, C. Wild, J. Lifson, and B. Moss.** 2001. Immunogenicity and protective efficacy of oligomeric human immunodeficiency virus type 1 gp140. J. Virol. **75:**645–653.
24. **Egan, 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 adults by a nonreplicating, host-range-restricted canarypox vector (ALVAC) carrying the HIV-1MN env gene. J. Infect. Dis. **171:**1623–1627.
25. **Emini, E. A., W. A. Schleif, J. H. Nunberg, A. J. Conley, Y. Eda, S. Tokiyoshi, S. D. Putney, S. Matsushita, K. E. 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.
26. **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.
27. **Etemad-Moghadam, 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 from infected monkeys. J. Virol. **72:**8437–8445.
28. **Farzan, M., H. Choe, E. Desjardins, Y. Sun, J. Kuhn, J. Cao, D. Archambault, P. 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 glycoprotein ectodomain. J. Virol. **72:**7620–7625.
29. **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.
30. **Gauduin, M. C., P. W. Parren, R. Weir, C. F. Barbas, 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.
31. **Graham, B. S., M. J. McElrath, R. I. Connor, D. H. Schwartz, G. J. Gorse, M. C. Keefer, M. J. Mulligan, T. J. Matthews, S. M. Wolinsky, D. C. Montefiori, S. H. Vermund, J. S. Lambert, L. Corey, R. B. Belshe, R. Dolin, P. F. Wright, B. T. Korber, M. C. Wolff, 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.
32. **Hanke, T., T. J. Blanchard, J. Schneider, C. M. Hannan, M. Becker, S. C. Gilbert, A. V. Hill, G. L. Smith, and A. McMichael.** 1998. Enhancement of MHC class I-restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination regime. Vaccine **16:**439–445.
33. **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. McMichael.** 1999. Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. J. Virol. **73:**7524–7532.
34. **Harada, S., Y. Koyanagi, and N. Yamamoto.** 1985. Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science **229:**563–566.
35. **Hasenkrug, K. J., and U. Dittmer.** 2000. The role of CD4 and CD8 T cells in recovery and protection from retroviral infection: lessons from the Friend virus model. Virology **272:**244–249.
36. **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. **Igarashi, T., Y. Endo, G. Englund, R. Sadjadpour, T. Matano, C. Buckler, A. Buckler-White, R. Plishka, 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 virus. Proc. Natl. Acad. Sci. USA **96:**14049–14054.
38. **Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho.** 1999. Dramatic rise in plasma viremia after $CD8^+$ T cell depletion in simian immunodeficiency virus-infected macaques. J. Exp. Med. **189:**991–998.
39. **Jonker, M., F. J. Nooij, P. van Suylichem, P. Neuhaus, and G. Goldstein.** 1986. The influence of OKT8F 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. Iliff, 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:**4218–4225.
41. **Kent, S. J., A. Zhao, S. J. Best, J. D. Chandler, D. B. Boyle, and I. A. Ramshaw.** 1998. Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of consecutive priming with DNA and boosting with recombinant fowlpox virus. J. Virol. **72:**10180–10188.
42. **Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, et al.** 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science **248:**1109–1112.
43. **Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, 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. **Lifson, J. D., J. L. Rossio, R. Arnaout, L. Li, T. L. Parks, D. K. Schneider, R. F. Kiser, V. J. Coalter, G. Walsh, R. J. Imming, B. Fisher, B. M. Flynn, N. Bischofberger, M. Piatak, Jr., V. M. Hirsch, M. A. Nowak, and D. Wodarz.** 2000. Containment of simian immunodeficiency virus infection: cellular immune responses and protection from rechallenge following transient postinoculation antiretroviral treatment. J. Virol. **74:**2584–2593.
45. **Lu, S., J. Arthos, D. C. Montefiori, Y. Yasutomi, K. Manson, F. Mustafa, E. Johnson, J. C. Santoro, J. Wissink, J. I. Mullins, J. R. Haynes, N. L. Letvin, M. Wyand, and H. L. Robinson.** 1996. Simian immunodeficiency virus DNA vaccine trial in macaques. J. Virol. **70:**3978–3991.
46. **Mascola, J. R., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. R. Brown, C. V. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, H. Katinger, and D. L. Birx.** 1999. Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J. Virol. **73:**4009–4018.
47. **Mascola, J. R., S. W. Snyder, O. S. Weislow, S. M. Belay, R. B. Belshe, D. H. Schwartz, M. L. Clements, R. Dolin, B. S. Graham, G. J. Gorse, M. C. Keefer, M. J. McElrath, M. C. Walker, K. F. Wagner, J. G. McNeil, F. E. McCutchan, D. S. Burke, and The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group.** 1996. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. J. Infect. Dis. **173:**340–348.
48. **Matano, T., R. Shibata, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin.** 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J. Virol. **72:**164–169.
49. **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.
50. **Metzner, K. J., X. Jin, F. V. Lee, A. Gettie, D. E. Bauer, M. Di Mascio, A. S. Perelson, P. A. Marx, D. D. Ho, L. G. Kostrikis, and R. I. Connor.** 2000. 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.
51. **Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, 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 monomeric gp120. J. Virol. **69:**101–109.
52. **Musey, 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:**1267–1274.
53. **Nishimura, Y., T. Igarashi, N. Haigwood, R. Sadjadpour, R. J. Plishka, A. Buckler-White, R. Shibata, and M. A. 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.
54. **Ogg, G. S., X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D. D. Ho, D. F. Nixon, and A. J. McMichael.** 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science **279:**2103–2106.
55. **Ourmanov, I., C. R. Brown, B. Moss, M. Carroll, L. Wyatt, L. Pletneva, S. Goldstein, D. Venzon, and V. M. Hirsch.** 2000. Comparative efficacy of recombinant modified vaccinia virus Ankara expressing simian immunodeficiency virus (SIV) Gag-Pol and/or Env in macaques challenged with pathogenic SIV. J. Virol. **74:**2740–2751.
56. **Pal, R., D. Venzon, N. L. Letvin, S. Santra, D. C. Montefiori, N. R. Miller, E. Tryniszewska, M. G. Lewis, T. C. VanCott, V. Hirsch, R. Woodward, A. Gibson, M. Grace, E. Dobratz, P. D. Markham, Z. Hel, J. Nacsa, M. Klein, J. Tartaglia, and G. Franchini.** 2002. ALVAC-SIV-gag-pol-env-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIV_{mac}-induced immunodeficiency. J. Virol. **76:**292–302.
57. **Parren, P. W., H. J. Ditzel, R. J. Gulizia, J. M. Binley, C. F. Barbas III, D. R. Burton, and D. E. Mosier.** 1995. Protection against HIV-1 infection in hu-PBL-SCID mice by passive immunization with a neutralizing human monoclonal antibody against the gp120 CD4-binding site. AIDS **9:**F1–F6.
58. **Parren, P. W., P. A. Marx, A. J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, and D. R. Burton.** 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J. Virol. **75:**8340–8347.
59. **Poignard, P., E. O. Saphire, P. W. H. I. Parren, and D. R. Burton.** 2001. gp120: biologic aspects of structural features. Annu. Rev. Immunol. **19:**253–274.
60. **Polacino, P. S., V. Stallard, J. E. Klaniecki, S. Pennathur, D. C. Montefiori, A. J. Langlois, B. A. Richardson, W. R. Morton, R. E. Benveniste, and S.-L. Hu.** 1999. Role of immune responses against the envelope and the core antigens of simian immunodeficiency virus SIVmne in protection against homologous cloned and uncloned virus challenge in macaques. J. Virol. **73:**8201–8215.
61. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent end points. Am. J. Hyg. **27:**493–497.
62. **Reimann, K. A., J. T. Li, R. Veazey, M. Halloran, I. W. Park, G. B. Karlsson, J. Sodroski, and N. L. Letvin.** 1996. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate *env* causes an AIDS-like disease after in vivo passage in rhesus monkeys. J. Virol. **70:**6922–6928.
63. **Reitter, J. N., R. E. Means, and R. C. Desrosiers.** 1998. A role for carbohydrates in immune evasion in AIDS. Nat. Med. **4:**679–684.
64. **Rinaldo, C., X. L. Huang, Z. F. Fan, M. Ding, L. Beltz, A. Logar, D. Panicali, G. Mazzara, J. Liebmann, M. Cottrill, et al.** 1995. High levels of anti-human immunodeficiency virus type 1 (HIV-1) memory cytotoxic T-lymphocyte activity and low viral load are associated with lack of disease in HIV-1-infected long-term nonprogressors. J. Virol. **69:**5838–5842.
65. **Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose.** 2001. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. Cell **106:**539–549.
66. **Rossio, J. L., M. T. Esser, K. Suryanarayana, D. K. Schneider, J. W. Bess, Jr., G. M. Vasquez, T. A. Wiltrout, E. Chertova, M. K. Grimes, Q. Sattentau, L. O. Arthur, L. E. Henderson, and J. D. Lifson.** 1998. Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. J. Virol. **72:**7992–8001.
67. **Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, J. Ghrayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann.** 1999. Control of viremia in simian immunodeficiency virus infection by $CD8^+$ lymphocytes. Science **283:**857–860.
68. **Seth, A., I. Ourmanov, M. J. Kuroda, J. E. Schmitz, M. W. Carroll, L. S. Wyatt, B. Moss, M. A. Forman, V. M. Hirsch, and N. L. Letvin.** 1998. Recombinant modified vaccinia virus Ankara-simian immunodeficiency virus gag pol elicits cytotoxic T lymphocytes in rhesus monkeys detected by a major histocompatibility complex class I/peptide tetramer. Proc. Natl. Acad. Sci. USA **95:**10112–10116.
69. **Seth, A., I. Ourmanov, J. E. Schmitz, M. J. Kuroda, M. A. Lifton, C. E. Nickerson, L. Wyatt, M. Carroll, B. Moss, D. Venzon, N. L. Letvin, and V. M. Hirsch.** 2000. Immunization with a modified vaccinia virus expressing simian immunodeficiency virus (SIV) Gag-Pol primes for an anamnestic Gag-specific cytotoxic T-lymphocyte response and is associated with reduction of viremia after SIV challenge. J. Virol. **74:**2502–2509.
70. **Shibata, R., M. D. Hoggan, C. Broscius, G. Englund, T. S. Theodore, A. Buckler-White, L. O. Arthur, Z. Israel, A. Schultz, H. C. Lane, et al.** 1995. Isolation and characterization of a syncytium-inducing, macrophage/T-cell line-tropic human immunodeficiency virus type 1 isolate that readily infects chimpanzee cells in vitro and in vivo. J. Virol. **69:**4453–4462.
71. **Shibata, R., T. Igarashi, N. Haigwood, A. Buckler-White, R. Ogert, W. Ross,**

R. Willey, M. W. Cho, and M. A. Martin. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV 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. Punt, 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. Bett, R. Youil, D. C. Kaslow, and E. A. Emini.** 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature **415:**331–335.
75. **Silvera, P., M. W. Richardson, J. Greenhouse, J. Yalley-Ogunro, N. Shaw, J. Mirchandani, K. Khalili, J. F. Zagury, M. G. Lewis, and J. Rappaport.** 2002. Outcome of simian-human immunodeficiency virus strain 89.6p challenge following vaccination of rhesus macaques with human immunodeficiency virus Tat protein. J. Virol. **76:**3800–3809.
76. **Stamatatos, L., and C. Cheng-Mayer.** 1998. An envelope modification that renders a primary, neutralization-resistant clade B human immunodeficiency virus type 1 isolate highly susceptible to neutralization by sera from other clades. J. Virol. **72:**7840–7845.
77. **Sullivan, N., Y. Sun, J. Li, W. Hofmann, and J. Sodroski.** 1995. Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T-cell line-passaged human immunodeficiency virus type 1 isolates. J. Virol. **69:**4413–4422.
78. **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.
79. **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.
80. **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.
81. **Wyatt, R., J. Moore, M. Accola, E. Desjardin, J. Robinson, and J. Sodroski.** 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.
82. **Wyatt, R., N. Sullivan, M. Thali, H. Repke, D. Ho, J. Robinson, M. Posner, and J. Sodroski.** 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.
83. **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.