Vectored Gag and Env but Not Tat Show Efficacy against Simian-Human Immunodeficiency Virus 89.6P Challenge in Mamu-A*01–Negative Rhesus Monkeys

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Simian-human immunodeficiency virus (SHIV) challenge studies in rhesus macaques were conducted to evaluate the efficacy of adenovirus-based vaccines in the context of different major histocompatibility complex class I genetic backgrounds and different vaccine compositions. Mamu-A*01 allele-negative rhesus monkeys were immunized with one of the following vaccine constructs: (i) replication-defective recombinant adenovirus type 5 (Ad5) expressing human immunodeficiency virus type 1 (HIV-1) Tat (Ad5/HIVTat); (ii) Ad5 vector expressing simian immunodeficiency virus (SIV) Gag (Ad5/SIVGag); (iii) Ad5 vector expressing the truncated HIV-1$_{jrfl}$ Env, gp140 (Ad5/gp140_jrfl); (iv) Ad5 vector expressing the SHIV-89.6P gp140 (Ad5/gp140_89.6P); or (v) the combination of Ad5/SIVGag and Ad5/gp140_jrfl. Following intravenous challenge with SHIV-89.6P, only those cohorts that received vaccines expressing Gag or Env exhibited an attenuation of the acute viremia and associated CD4-cell lymphopenia. While no prechallenge neutralizing antibody titers were detectable in either Ad5/gp140-vaccinated group, an accelerated neutralizing antibody response was observed in the Ad5/gp140_89.6P-vaccinated group upon viral challenge. The set-point viral loads in the Ad5/SIVGag- and Ad5/gp140_jrfl-vaccinated groups were associated with the overall strength of the induced cellular immune responses. To examine the contribution of Mamu-A*01 allele in vaccine efficacy against SHIV-89.6P challenge, Mamu-A*01-positive monkeys were immunized with Ad5/SIVGag. Vaccine-mediated protection was significantly more pronounced in the Mamu-A*01-positive monkeys than in Mamu-A*01-negative monkeys, suggesting the strong contributions of T-cell epitopes restricted by the Mamu-A*01 molecule. The implications of these results in the development of an HIV-1 vaccine will be discussed.

A human immunodeficiency virus type 1 (HIV-1) vaccine should ideally be able to elicit potent, broadly cross viral neutralizing antibody responses and/or cytotoxic T-cell responses (20, 30, 45). Passive immunization by adoptive transfer of neutralizing antibodies has been shown to prevent infection and/or disease in both rhesus monkey simian-human immunodeficiency virus (SHIV) and in chimpanzee HIV-1 challenge models (13, 24, 25, 31, 32, 36, 42, 44, 53). These studies established that neutralizing antibodies can protect against AIDS virus infection. However, due to the high viral variability and the innate ability of HIV-1 to evade antibody-mediated neutralization (19, 40, 51), efforts to develop such a vaccine to elicit neutralizing antibody responses have not been successful (4, 10, 50).

In contrast to the antibody approach, promising results have been observed from studies using vaccines designed to elicit virus-specific cytotoxic T-lymphocyte (CTL) responses. In these studies, viral vectors or DNA vaccines were used to induce virus-specific CTL responses. Following pathogenic SHIV or simian immunodeficiency virus (SIV) challenges, the vaccinated monkeys experienced reduced acute viremia and/or exhibited long lasting control of chronic viremia and diseases progression (3, 12, 17, 37, 41, 46, 48).

Important considerations in the development of an HIV-1 vaccine are to determine the antigen composition of the vaccine and the contributions of each component to vaccine efficacy. Internal viral antigens, such as Gag and Pol, have been demonstrated to be potent vaccine components for eliciting T-cell responses (2, 3, 9, 46). The value of the envelope protein (Env) in a CTL-based vaccine is less well defined. Several studies previously suggested that inclusion of Env in vectored multicomponent vaccine was able to confer protection against SHIV challenge (2, 34, 39). However, since these studies employed Env antigens homologous to the challenge virus, the contribution of Env-induced CTL responses to the vaccine efficacy could not be differentiated from the potential contribution of homologous neutralizing antibody responses. More recently, it has been reported that combining a heterologous Env antigen with the Gag-Pol-Nef vaccine was able to improve protection against SHIV challenge, and the improved protec-

tion was associated with anamnestic cellular immune responses (21). The study, therefore, provided direct evidence of the value of including Env in CTL-based vaccines. Nonetheless, the potency of a heterologous Env, relative to other viral antigens, still remains to be defined and will be important in defining a final vaccine composition.

The early regulatory protein Tat represents another potential vaccine target. Tat-specific antibody or CTL responses have been implicated in association with slow progression of HIV-1 infection (49, 54). Tat vaccine studies in nonhuman primate viral challenge models have resulted in conflicting results, ranging from no protection to significant, long-term protection (1, 5, 6, 7, 22, 47, 27, 38). The differences in immunization regimen, viral stock, route of viral challenge, and animal species could have been responsible for the different outcomes.

Replication-incompetent recombinant adenovirus type 5 (Ad5) vectors expressing SIV Gag have been reported to elicit a potent CTL response in major histocompatibility complex (MHC) class I Mamu-A*01-positive rhesus monkeys and confer effective protection against disease following pathogenic SHIV-89.6P challenge (46). CTL responses elicited by the Gag vaccine were chiefly focused on a single Mamu-A*01-restricted epitope, Gag$_{181-189}$CM9 (CM9). Since expression of Mamu-A*01 molecules has been shown to be associated with better control of viremia and/or attenuation of disease progression in SIV and SHIV infections (28, 29, 33, 35, 55), it has become necessary to also evaluate the vectored Gag vaccine in Mamu-A*01-negative monkeys.

In an attempt to address these issues, several SHIV-89.6P challenge studies were conducted, which evaluated the efficacy of the Ad5 vector expressing SIV Gag (Ad5/SIVGag), heterologous HIV-1 Env (Ad5/gp140_jrfl), homologous HIV-1 Env (Ad5/gp140_89.6P), or HIV-1 Tat (Ad5/HIVTat) in Mamu-A*01-negative macaques. In addition, the efficacy in the Mamu-A*01-negative monkeys was compared with that achieved by Ad5/SIVGag in Mamu-A*01-positive monkeys. The results showed the following: (i) the *Mamu-A**01 allele confers a genetic advantage for Gag-based vaccines in this challenge model; (ii) a heterologous Env vaccine, used either alone or in combination with the Gag vaccine, can confer protection against viral challenge of Mamu-A*01-negative monkeys by inducing cross-reactive, Env-specific T-cell responses; (iii) compared to a heterologous Env antigen, a homologous Env vaccine can additionally prime for neutralizing antibody responses leading to a more effective protection against the viral challenge; and (iv) Tat vaccine delivered by the Ad5 vector failed to show protection against the SHIV-89.6P challenge in rhesus monkeys.

MATERIALS AND METHODS

Vaccines. Genes coding for SIV$_{mac239}$ Gag, HIV-1$_{jrfl}$ Tat, HIV-1$_{jrfl}$ Env gp140 (amino acid residues 1 to 683 per HXB2 numbering), and SHIV-89.6P clone KB9 gp140 (amino acid residues 1 to 670 per HXB2 numbering) were synthesized based on codons frequently used in mammalian cells (46). Methods for construction of the E1- and E3-deleted, replication-incompetent, recombinant adenovirus type 5 (expressing the respective viral genes) were described previously (46). The synthetic genes were cloned into the BglII site located between the human cytomegalovirus promoter (with intron A) and the bovine growth hormone polyadenylation signal of the adenoviral shuttle vector pHCMVIB-GHpA1. The shuttle vector was recombined with an adenoviral backbone plasmid, pAd-E1E3, which contains the complete Ad5 genome except for the E1 and E3 regions. This plasmid was then transfected into 293 cells to generate recombinant viruses. For vaccine use, the recombinant adenovirus was further amplified in 293 cells, and then purified by cesium chloride gradient centrifugation. Viral particles were quantitated based on the nucleic acid content via the Maizel spectroscopic method (23).

Animal studies. Indian rhesus monkeys (*Macaca mulatta*) were maintained in accordance with the institutional animal care protocols of Merck Research Laboratories and New Iberia Research Center. Monkeys were typed for expression of the *Mamu-A**01 allele according to established PCR methods (46). For immunization, vaccine was suspended in 1 ml of phosphate-buffered saline (PBS). The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered intramuscularly, in 0.5-ml aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Sera and peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at specified time points during the immunization regimen. Viral challenge was carried out by intravenous injection of 50 50% median infectious doses (MID$_{50}$) of cell-free SHIV-89.6P (46). Monkeys were monitored for clinical signs of disease progression and cared for under the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

IFN-γ ELISPOT assay. Ninety-six-well, flat-bottomed plates (Millipore, Immobilon-P membrane) were coated with 1 μg/well of anti-gamma interferon (IFN-γ) monoclonal antibody MD-1 (U-Cytech-BV) overnight at 4°C. The plates were then washed three times with PBS and blocked with R10 medium (RPMI, 0.05 mM 2-mercaptoethanol, 1 mM sodium pyruvate, 2 mM L-glutamate, 10 mM HEPES, 10% fetal bovine serum) for 2 h at 37°C. The medium was discarded from the plates, and freshly isolated PBMC were added at 2×10^5 to 4×10^5 cells/well. The cells were stimulated in the absence (mock) or presence of the HIV-1 peptide pool (4 μg/ml per peptide) or concanavalin A (Sigma Chemical) per ml. Pools consisting of 20-amino-acid peptides, shifted by 10 amino acids (Synpep, CA), were constructed from entire SIVmac239 Gag, SIVmac239 Nef, SHIV-89.6P gp140, or HIV-1jrfl Tat sequences. The CM9 peptide was synthesized in a likewise manner. Cells were then incubated for 20 to 24 h at 37°C in 5% CO$_2$. Plates were washed six times with PBST (PBS plus 0.05% Tween 20), 100 μl/well of a 1:400 dilution of anti-IFN-γ polyclonal biotinylated detector antibody solution (U-Cytech-BV) was added, and the plates were incubated overnight at 37°C. The next day, the plates were washed six times with PBST. Color was developed by incubating in nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate (NBT-BCIP) (Pierce) for 10 min. Spots were counted under a dissecting microscope and data were reported as numbers of spot-forming cells (SFC) per million PBMC.

Flow cytometric assays. Intracellular staining for IFN-γ production was conducted following a previously established protocol (8). The methods for preparing CM9 tetramer reagent and staining lymphocytes have been described elsewhere (43). Samples were analyzed by flow cytometry on a FACScalibur (Becton Dickinson). Gated CD3$^+$ CD8$^+$ T cells were examined for staining with tetrameric Mamu-A*01–CM9 complex. For each sample, 30,000 gated CD3$^+$ CD8$^+$ T-cell events were collected and analyzed on CellQuest program (Becton Dickinson), and the results were presented as the percent of tetramer-positive cells within CD3$^+$- and CD8$^+$-positive events.

ELISA. Env and Tat binding antibody titers were determined by enzyme-linked immunosorbent assay (ELISA). For Tat ELISA, 96-well plates were coated with 50 μl/well of a recombinant HIV-1 IIIB Tat protein at a concentration of 2 μg/ml at 4°C overnight. For Env ELISA, culture supernatants from 293 cells transiently transfected with V1Jns/HIV-1$_{jrfl}$ gp140 or V1Jns/SHIV-89.6P gp140 were used as the source of antigen. The Env protein concentration in the supernatant was quantitated by a sandwich ELISA, using purified gp140 as standard. The 96-well plates were coated with 50 μl/well an HIV-1 gp120 C terminus-specific goat antibody (D7324, Cliniqa, Fallbrook, CA) at a concentration of 2 μg/ml at 4°C overnight; 100 μl of transfected supernatant containing 1 μg gp140 per ml was added to each well, which was followed by incubation at room temperature for 1 h. After three washes with PBST, the plates were blocked with 3% skim milk in PBST.

Test samples were diluted in PBST containing 3% milk, and added to the plate at 100 μl per well. After an additional 1-hour incubation, plates were washed as above and incubated with 50 μl per well of horse radish-conjugated goat anti-human immunoglobulin G. This was followed by addition of o-phenylenediamine dihydrochloride substrate (Sigma). The colors that developed were read in an ELISA reader at an optical density of 490 nm. The antibody titers were defined as reciprocals of the highest dilutions which yield optical density at 490 nm values higher than two times that of conjugate controls.

Neutralizing antibody assay. Viral neutralization assays were conducted using methods previously published (11). CEMX174 human T cells were infected at a

TABLE 1. Immunization schedules for the three SHIV-89.6P challenge studies

Group	Monkeys	Vaccine (viral particles)	Immunizations (wk)	Challenge (wk)
Study A (Mamu-A*01-negative)				
1	00D001, 00C008, 00C056, 00D005	5×10^{10} Ad5/SIV Gag + 5×10^{10} empty Ad5	0, 4 and 29	41
2	00C065, 00C068, 00C070, 00D004	5×10^{10} Ad5/gp140_jrfl + 5×10^{10} empty Ad5	0, 4 and 29	41
3	00C071, 00C073, 00C074, 00D003	5×10^{10} Ad5/SIV Gag + 5×10^{10} Ad5/gp140_jrfl	0, 4 and 29	41
4	00D008, 00D014, 00D041, 00C232	5×10^{10} Ad5/gp140_89.6P + 5×10^{10} empty Ad5	14 and 29	41
5	00D009, 00D086, 00D013, 00D137	None	N/A[a]	41
Study B (Mamu-A*01-negative)				
1	CB86, CB84, CB8T, CB93	10^{11} Ad5/HIV Tat	0, 4 and 28	49
2	CC1V, CC1R, CC64, CC7E	10^{11} empty Ad5	0, 4 and 28	49
Study C (Mamu-A*01-positive)				
1	AW2D, AW2T, AW2W	10^7 Ad5/SIV Gag	0	6
2	99C048, 145T, 126H	10^{11} Ad5/SIV Gag	0	6
3	AW9C, CB8P, CC7J, AW80, 89Q, AW35	None	N/A	6
	040F, 97N114	10^{11} empty Ad5	0	6

[a] N/A, not applicable.

multiplicity of infection of ca. 0.01. After overnight incubation, cells were washed with medium and plated into 96 well plates. Test sera were diluted by twofold serial dilutions and mixed with the cells. Cultures were incubated an additional 72 h and then assayed for viral production by a commercial SIV viral core p27 assay kit (Coulter Immunology). Endpoint titers were recorded as the reciprocal of the serum dilution in which ≥95% of the viral antigen production was inhibited compared to untreated viral growth control wells.

Quantitation of plasma viral load. Plasma viral load was measured by a modified version of the ROCHE AMPLICOR UtraSensitive Assay referred to as SIV UltraSensitive Real-Time PCR assay with a quantification limit of 50 viral RNA copies/ml. The assays were conducted by Consolidated Laboratory Services, Van Nuys, CA.

Statistical analyses. Comparisons of cohort immune or virological parameters were performed by calculating the ratios of the cohort geometric means and the associated 95% confidence intervals (95% CI); confidence intervals not overlapping with unity suggest a statistically significant difference. In certain cases, comparisons between pairs of data sets were made using standard t test with equal variances. Correlates of disease protection were determined by associating prechallenge predictor variables and postchallenge viral load values. Spearman's Rho, a nonparametric and robust rank-based statistical test (16), was used to detect trends of monotonic relationships between viral load data and these predictor candidates. Data analyses were carried out using JMP 5.0.1 software (SAS Institute, Cary, NC).

RESULTS

Vaccination and SHIV-89.6P challenge. Three separate studies were conducted (Table 1). In the first study (study A), 20 Mamu-A*01-negative rhesus monkeys were divided into five groups of four monkeys each, including four vaccine groups and one naive control group. The vaccine groups were immunized with 5×10^{10} viral particles of Ad5/SIVGag, 5×10^{10} viral particles of Ad5/gp140_jrfl, 5×10^{10} viral particles of Ad5/SIVGag plus 5×10^{10} viral particles of Ad5/gp140_jrfl, or 5×10^{10} viral particles of Ad5/gp140_89.6P. To ensure that all animals receive same dose of virus particles, the vaccines with single vector were supplemented with 5×10^{10} viral particles of empty viral vector. For the groups receiving Ad5/SIVGag, Ad5/gp140_jrfl or Ad5/SIVGag plus Ad5/gp140_jrfl, the immunizations were given three times at weeks 0, 4, and 29. For the group receiving Ad5/gp140_89.6P, the immunizations were

given twice at weeks 14 and 29. All monkeys were challenged intravenously 12 weeks after the final immunization with 50 MID$_{50}$ of SHIV-89.6P.

In the second study (study B), a cohort of four Mamu-A*01-negative macaques was given three immunizations of 10^{11} viral particles of Ad5/HIV Tat, while another cohort was administered three doses of 10^{11} viral particles of empty Ad5 vectors. At 21 weeks following the last immunization, all monkeys were challenged with the same stock of SHIV-89.6P virus. In the third study (study C), six Mamu-A*01-positive monkeys were immunized with a single injection of 10^7 viral particles ($n = 3$) or 10^{11} viral particles of Ad5/SIVGag ($n = 3$). Eight Mamu-A*01-positive monkeys served as nonvaccinated controls. Among the controls, two monkeys were given a single injection of 10^{11} viral particles of empty vectors. Six weeks after the immunization, all monkeys were challenged intravenously with 50 MID$_{50}$ of SHIV-89.6P.

Vaccine-induced immunity. In Mamu-A*01-negative monkeys, the Gag and Env vaccines elicited significant specific T-cell responses as measured by ELISPOT assay (Fig. 1). Overall, the levels of responses elicited by the Gag vaccine were comparable to those elicited by the Env vaccines. Preferential responses to the Env antigen homologous to the administered vaccine were evident. However, significant crossreactive cellular immunity was observed between the two Env vaccines, which share approximately 80% homology in amino acid sequence. Monkeys receiving Ad5/gp140_jrfl vaccine, either alone or in combination with Ad5/SIVGag, developed both HIV-1$_{jrfl}$ Env and SHIV-89.6P Env-specific responses. Similarly, monkeys receiving Ad5/gp140_89.6P also responded to both Env vaccines. The monkeys receiving both Ad5/SIVGag and Ad5/gp140_jrfl had Gag- and Env-specific responses, similar to those seen with monkeys receiving either vaccine alone. This indicates that the combination of Gag and Env does not compromise the potency of the individual vaccine
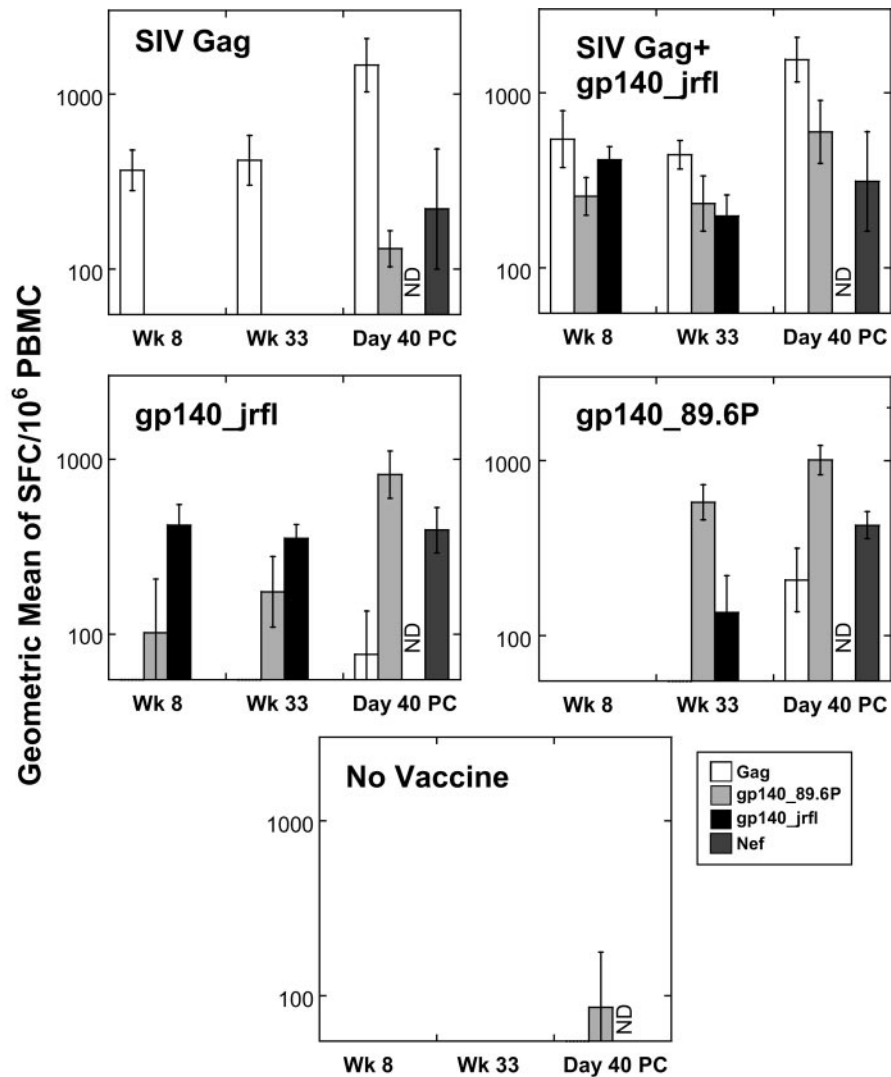
FIG. 1. Antigen-specific T-cell responses in monkeys immunized with SIV Gag and gp140 antigens delivered using Ad5 vectors (study A). The data are reported as the cohort geometric means of SFC/10⁶ PBMC against the defined peptide pools and the associated standard errors of the geometric means. Shown are the results at 4 weeks after the second vaccine dose (week 8), 4 weeks after the third dose (week 33), around the time of challenge (week 40), and 40 days after virus challenge (day 40 PC). Postchallenge responses against the gp140_jrfl pool were not determined (ND).

components, while broadening the overall virus-specific T-cell responses.

The specific T-cell responses, as determined by intracellular cytokine staining, consisted of both CD4$^+$ and CD8$^+$ cell populations, of which the CD8$^+$ cells constitute about 65 to 85% of total antigen-specific CD3-positive T cells. There was no overt difference in the ratios of CD4$^+$ and CD8$^+$ population among the different vaccine groups.

Significant Tat-specific T-cell responses were observed in the Ad5/HIVTat immunized macaques prior to virus challenge; three of the four monkeys (CB86, CB8T, and CB93) developed Tat T-cell responses within the range exhibited by those immunized with Gag or Env vaccines (Table 2).

With respect to Mamu-A*01-positive monkeys, both Ad5/SIVGag vaccinated groups developed significant Gag-specific SFC responses (Fig. 2). Consistent with previous findings (46),

the Gag-specific T-cell responses in the Mamu-A*01-positive monkeys were highly focused on the CM9 CD8$^+$ epitope. Detectable levels of CM9-specific responses were also confirmed by tetramer staining (data not shown).

Viremia and CD4 cell lymphopenia. Control animals in the studies, with the exception of CC7E, exhibited severe viremia (Fig. 3) and acute CD4 cell lymphopenia (not shown) similar to those previously reported for SHIV-89.6P infection (3, 46). Peak viremia occurred approximately two weeks post challenge, with the cohort geometric mean plasma viral loads of 1.1×10^8 and 1.3×10^8 copies/ml for the Mamu-A*01-positive ($n = 8$) and Mamu-A*01-negative controls ($n = 8$), respectively; this difference was not statistically significant. The set-point viral loads, taken as the geometric mean of the viral loads recorded between months 2 and 4, were 3.8×10^5 and 4.5×10^5 copies/ml for the Mamu-A*01-positive and Mamu-A*01-

TABLE 2. Binding antibody titers and levels of specific T-cell responses against Tat in study B[a]

Group and monkey no.	Anti-Tat titer						SFC/10⁶ PBMC					
	Prechallenge			Postchallenge			Wk 8		Wk 49		Day 24 Postchallenge	
	Wk-2	Wk 27	Wk 30	Day 17	Day 27	Day 40	Mock	Tat	Mock	Tat	Mock	Tat
Ad5/HIV Tat vaccinees												
CB86	<100	<100	300	100	100	100	0	790	6	238	5	276
CB84	<100	100	900	100	100	100	1	30	1	5	0	5
CB8T	<100	900	900	900	2700	900	14	108	8	26	0	2
CB93	<100	300	900	100	300	300	4	535	1	28	2	172
Controls												
CCIV	<100	100	100	<100	<100	<100	29	15	0	1	0	0
CC1R	<100	300	300	100	100	<100	3	5	1	0	2	0
CC64	<100	<100	<100	<100	<100	<100	0	4	1	1	0	2
CC7E	<100	<100	<100	<100	<100	<100	5	9	1	5	2	2

[a] ELISPOT responses were measured using full Tat peptide pool and mock control.

negative controls, respectively; this difference was not statistically significant. All animals except CC7E experienced profound acute CD4 cell loss; by day 18 after challenge all had CD4 counts dropped below 100 cells μl^{-1} (not shown).

In contrast to the controls, the vaccinated cohorts (except for Ad5/HIVTat) showed various degrees of mitigation of both viral replication and CD4 cell lymphopenia. With regards to the Mamu-A*01-negative monkeys, the vaccinated groups (with exception of the Ad5/HIVTat cohort) had geometric mean peak plasma viral loads 2- to 30-fold lower than that of the control cohort (see Table 3); however, the differences from those of the control group were significant only for the Ad5/SIVGag+Ad5/gp140_jrfl (14-fold [95% CI of 1.3- to 150-fold]), and Ad5/gp140_89.6P cohorts (31-fold [95% CI of 2.9- to 340-fold]). With the exception of the Ad5/HIVTat cohort, the set-point viral loads of the vaccinated animals were 30- to 100-fold lower than those of the nonvaccinated control group; differences from those of the control group were significant only for the Ad5/SIV Gag+Ad5/gp140_jrfl (110-fold [95% CI of 1.9- to 5800-fold]) and Ad5/gp140_89.6P cohorts (98-fold (95% CI of 1.1- to 8,500-fold]). Differences in geometric mean viral loads (peak or set-point) for any given pair of vaccine groups (excluding the Ad5/HIVTat cohort) were not statistically significant. The peak and set-point viral loads for the Ad5/HIVTat group (4.4×10^8 and 1.0×10^6 copies/ml, respectively) were both marginally higher than, though not statistically significant, from those of the control cohort.

The vaccinated groups also showed considerable mitigation of CD4 cell loss compared to that of the control group (Fig. 4). The postacute levels of circulating CD4 T cells, taken as the geometric means of the CD4 counts over the second to fourth month, negatively correlated with the peak viral loads on an animal-to-animal basis (Fig. 2). Mamu-A*01-negative macaques with peak viral loads above 5×10^7 copies per ml had CD4 levels in this period ≥70% lower compared to prechallenge levels. The CD4 counts for two SIV Gag-vaccinated monkeys (00C056 and 00D005) eventually recovered to the prechallenge levels at 1 year postchallenge, while the CD4 counts of a gp140_89.6P-vaccinated macaque (00D041) continued to decline to as low as 1% of the original levels. Overall, on the basis of the viremia and CD4 cell profiles, the five vaccines showed a descending order of the protection: Ad5/
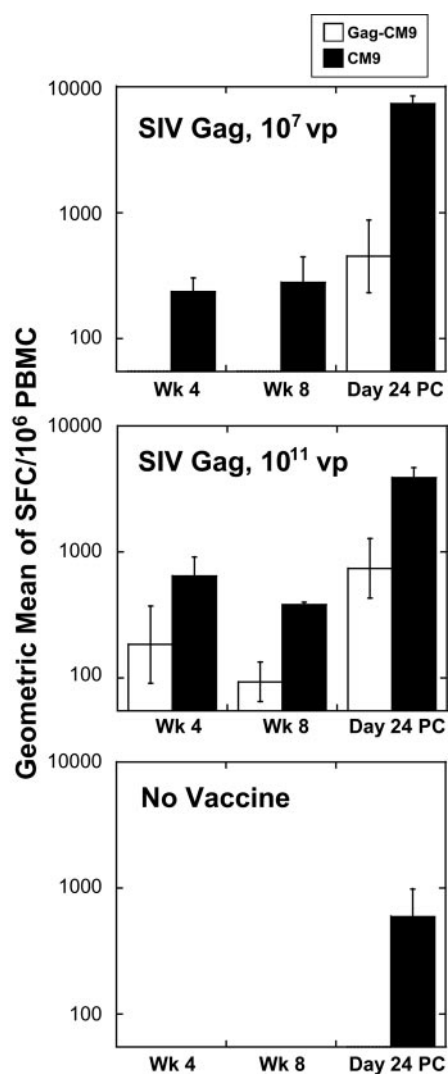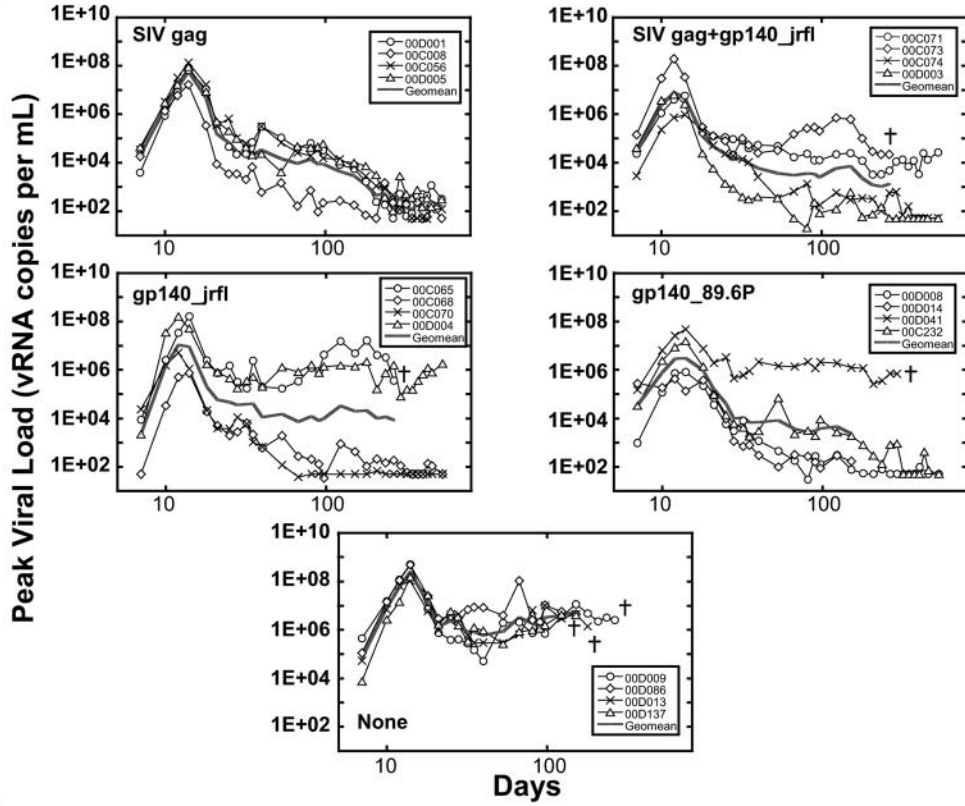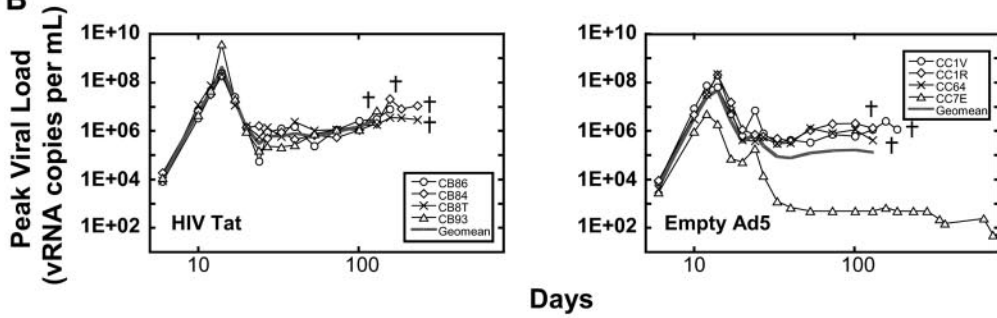

FIG. 2. Gag-specific T-cell responses in the Mamu-A*01-postive monkeys (study C). The data are reported as the cohort geometric means of the SFC/10⁶ PBMC against the CM9 peptide (CM9) and the Gag peptide pool excluding peptides containing the CM9 epitope (Gag-CM9); also shown are the associated standard errors of the geometric means. Shown are the results at 4 weeks after the vaccine dose (week 4), at the time of challenge (week 6), and 24 days after virus challenge (day 24 PC).
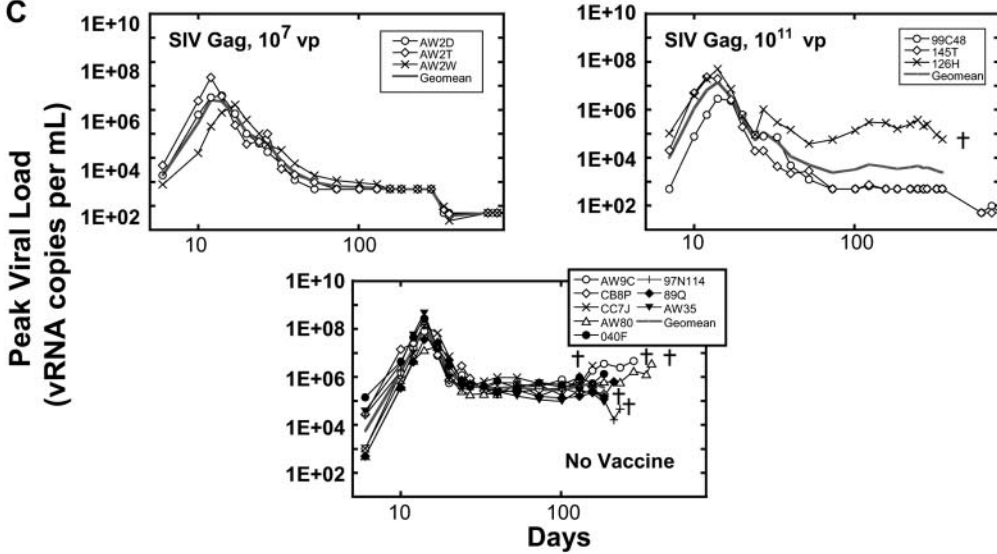
FIG. 3. Viral loads following intravenous challenge with SHIV-89.6P. The gray lines represent cohort geometric means of the viral loads up to when an animal's data point is unavailable. The animals that were humanely euthanized after exhibiting significant AIDS-like disease symptoms are marked. In study C, after day 300 the drop of baseline viral loads in the vaccine groups was due to the improved assay sensitivity to 50 viral RNA copies/ml.

TABLE 3. Cohort geometric means of the postchallenge virological parameters and the intercohort ratios (relative to those of the control cohorts) along with the 95% confidence intervals (in parentheses)

| Cohort and vaccine	Geometric means[a]		Ratios (higher/lower) of cohort geometric means			
			Peak VL		Set-point VL	
	Peak VL	Set-point VL	A*01[-] control	A*01[+] control	A*01[-] control	A*01[+] control
Mamu-A*01-negative						
Ad5/SIV Gag	6.0×10^7	1.2×10^4	2.1 (0.4, 13)		48 (1.0, 1400)	
Ad5/gp140_jrfl	1.8×10^7	1.4×10^4	7.1 (0.5, 96)		32 (0.2, 4200)	
Ad5/SIV Gag + Ad5/gp140_jrfl	9.2×10^6	4.2×10^3	14 (1.3, 150)		110 (1.9, 5800)	
Ad5/gp140_89.6P	4.0×10^6	4.6×10^3	31 (2.9, 40)		98 (1.1, 8500)	
Ad5/HIV Tat	4.4×10^8	1.0×10^6	3.5 (0.5, 25)		2.4 (0.1, 61)	
Control (n = 8)	1.3×10^8	4.5×10^5		1.2 (0.3, 4.5)		1.2 (0.1, 11)
Mamu-A*01-positive						
Ad5/SIV Gag, 10⁷ vp	5.3×10^6	6.9×10^3		21 (3.7, 120)		55 (6.9, 430)
Ad5/SIV Gag, 10î1 vp	1.5×10^7	8.8×10^2		7.4 (1.3, 44)		430 (220, 820)
Control (n = 8)	1.1×10^8	3.8×10^5	1.2 (0.3, 4.5)		1.2 (0.1, 11)	

[a] VL, viral load.
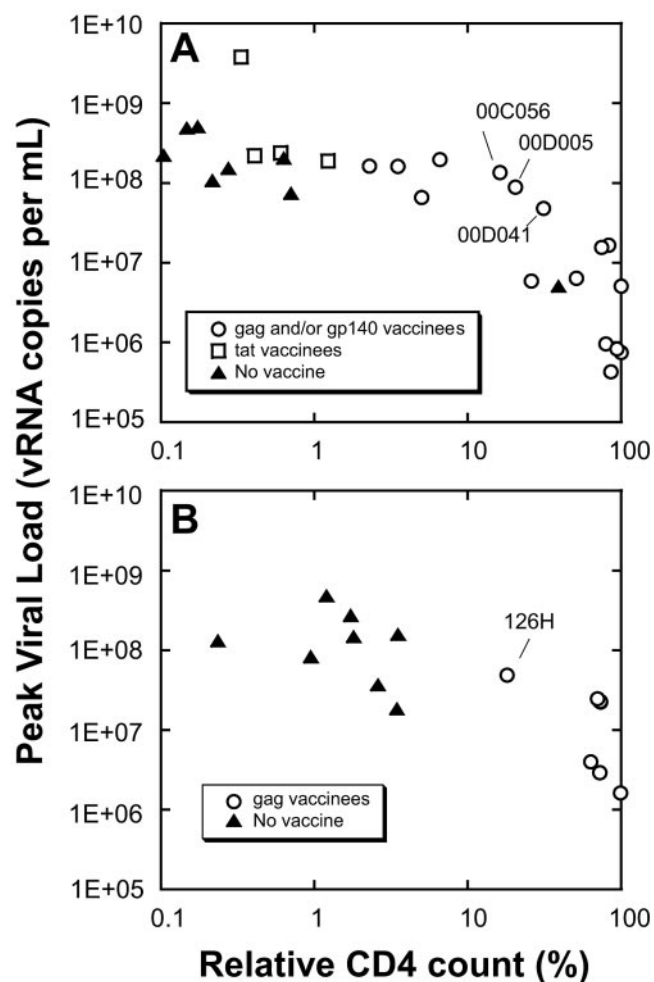
FIG. 4. Relationship between peak viral loads and postacute CD4 counts for (A) Mamu-A*01-negative and (B) Mamu-A*01-positive monkeys in all three studies.

gp140_89.6P > the combination of Ad5/SIVGag and Ad5/gp140_jrfl > Ad5/gp140_jrfl ≈ Ad5/SIVGag. There was no discernible protection with Ad/HIVTat vaccine.

Single immunization with either 10^7 or 10^{11} viral particles of Ad5/SIVGag offered significant protection in Mamu-A*01-positive monkeys (Fig. 3). Among the six monkeys in the two cohorts, five had normal CD4 counts and one (monkey 126H) had transient CD4 loss below 100 μl^{-1} (Fig. 4B). This represents a higher degree of protection than those observed in Mamu-A*01-negative monkeys, which received more injections of the same Ad5/SIVGag. The Mamu-A*01-positive vaccinees (n = 6) had approximately sevenfold lower peak viral loads (8.8×10^6 copies/ml versus 6.0×10^7 copies/ml) and about fivefold lower set-point viral loads (2.5×10^3 copies/ml versus 1.2×10^4 copies/ml) than did the Mamu-A*01 negative vaccinees; the former was a statistically significant difference.

Virus-specific immunity following the challenge. Vaccine-specific T-cell responses were drastically boosted following the viral challenge among the Gag and Env-vaccinated groups (Fig. 1 and 2). All vaccinated groups also developed comparable levels of cellular responses to Nef (a nonvaccine viral antigen), whereas they exhibited preferential, high-level responses to the vaccine components that each received. The heterologous Env vaccine, Ad5/gp140_jrfl, effectively primed for cross-reactive 89.6P Env-specific cellular immune responses upon viral challenge. Specifically, the groups receiving the Ad5/gp140_jrfl alone or in combination with Ad5/SIVGag showed elevated levels of 89.6P Env-specific T-cell responses of 817 and 596 SFC per million PBMC, respectively. This compares favorably with that in the monkeys receiving the homologous Env vaccine, at 1,006 SFC per million PBMC. In the Gag-vaccinated Mamu-A*01-positive monkeys, both CM9 and non-CM9 Gag responses expanded with viral challenge. In contrast to the vaccinated monkeys, the naive control monkeys had much lower overall responses to both vaccine and nonvaccine antigens. Tat-specific T-cell responses in the Ad5/HIVTat vaccinees did not expand upon viral challenge (Table 2). Also, low levels of Tat binding antibodies were detected prior to challenge but the levels did not improve after viral challenge

TABLE 4. Anti-SHIV-89.6P neutralizing titers in macaques of study A before and after viral challenge

Vaccine	Monkey no.	SHIV-89.6P neutralizing titer				
		Prechallenge (wk 36)	Day postchallenge:			
			12	28	40	67
Ad5/SIVGag	00D001	<10	<10	10	<10	<10
	00C008	<10	<10	<10	40	320
	00C056	<10	<10	40	640	1,280
	00D005	<10	<10	<10	1,280	320
Ad5/gp140_jrfl	00C065	<10	<10	<10	<10	<10
	00C068	<10	<10	<10	10	160
	00C070	<10	<10	<10	<10	40
	00D004	<10	<10	<10	<10	80
Ad5/SIVGag + Ad5/gp140_jrfl	00C071	<10	<10	20	160	640
	00C073	<10	<10	<10	10	640
	00C074	<10	<10	<10	20	20
	00D003	<10	<10	20	320	1,280
Ad5/gp140_89.6P	00D008	<10	<10	80	160	80
	00D014	<10	<10	320	640	320
	00D041	<10	<10	80	80	80
	00C232	<10	<10	80	80	80
None	00D009	<10	<10	<10	<10	<10
	00D086	<10	<10	<10	<10	<10
	00D013	<10	<10	<10	<10	<10
	00D137	<10	<10	<10	<10	<10

(Table 2). Nonvaccinated control animals did not develop any Tat-specific humoral or cellular immunity upon viral infection.

No SHIV-89.6P neutralizing antibodies were detectable prior to viral challenge in either heterologous or homologous Env vaccine groups (Table 4). However, SHIV-89.6P Env-specific binding antibodies were detected by ELISA prior to challenge in the gp140_89.6P vaccinated cohort and not in the gp140_jrfl vaccinated animals. Following viral challenge, all four monkeys in the group receiving Ad5/gp140_89.6P developed significant neutralizing titers on day 28. In contrast, monkeys receiving the Ad5/gp140_jrfl, did not develop significant neutralizing titers until day 67, at which time three monkeys had titers between 40 and 160 and one (00C065) remained negative. For the monkeys that received the Ad5/SIVGag, either alone or in combination with Ad5/gp140_jrfl, all except for one monkey (00D001) developed neutralizing titers by day 40. All naive monkeys failed to develop neutralizing antibody responses. This suggests that (i) a memory neutralizing response likely existed in the gp140_89.6P vaccinated animals prior to challenge and (ii) the control conferred initially by heterologous Env vaccine and Gag enabled the animals to develop virus neutralizing activity for additional protective efficacy.

Correlates of efficacy. Correlation analyses were performed using pairing of three prechallenge immune measurements (maximum at any given time, maximum postboost, and time-of-challenge ELISPOT responses against Gag and/or 89.6P Env) with two postchallenge parameters (peak- and postacute viral load) on an animal-to-animal basis. Data on the control animals and Ad5/gp140_89.6P vaccinees were excluded from the analyses, as the former would have unnecessarily biased the trends and the latter was complicated by the neutralizing an-

tibody immunity afforded by the vaccine. Importantly, a significant correlation was found between the maximum prechallenge cumulative ELISPOT responses (against Gag plus 89.6P Env) and the postacute viral load levels in the Manu-A*01-negative monkeys ($P < 0.03$; Fig. 5). No correlation could be established within the Mamu-A*01-positive animals due to the low viremia observed in the vaccine groups.
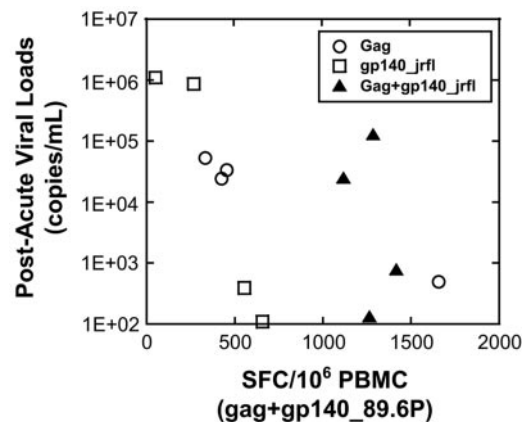

FIG. 5. Correlation of postacute viral loads with total ELISPOT responses (against Gag plus gp140_89.6P peptide pools) at peak any-time prior to challenge for study A vaccinees. A rank correlation analysis of the two parameters revealed a coefficient of −0.56 and one-tailed P of <0.03.

DISCUSSION

The studies presented here were designed to address (i) the effect of MHC class I genetic background on the vaccine efficacy and its implication in the SHIV monkey challenge model; (ii) the contribution of heterologous and homologous Env components to the efficacy of CTL-based vaccine and the mechanism involved; and (iii) the potency of Tat as a vaccine component relative to other viral antigens.

The MHC allele-associated protection has been documented for AIDS virus infections. For example, expression of HLA-B*27 and HLA-B*57 molecules was found to be associated with slow disease progression in HIV-1-infected individuals (14, 15, 18, 26). Expression of Mamu-A*01, –B*17, and/or -B*29 molecules was associated with either better control of viral replication or slower disease progression in SIV- or SHIV-infected rhesus monkeys (28, 29, 33, 35, 55). The dominant CTL responses to the viral epitopes restricted by the specific MHC class I molecules are believed to be responsible for disease attenuation (33). Consistent with this premise, we found that Ad/Gag-vaccinated Mamu-A*01-positive monkeys, with potent CTL responses to the Mamu-A*01-restricted CM9 epitope, exhibited better control of viral replication than did the Mamu-A*01-negative monkeys. We observed that plasma viral loads in the naive Mamu-A*01-positive and negative monkeys were not significantly different. However, it was previously observed that there was a profound difference in the viral loads in the lymph nodes between Mamu-A*01-positive and negative monkeys during the acute phase of SHIV-89.6P infection (55). The differential protective effects of a vaccine in Mamu-A*01-negative and Mamu-A*01-positive monkeys may have practical implications in conducting vaccine efficacy studies with the pathogenic SHIV-89.6P challenge model. For instance, Mamu-A*01-negative monkeys, which exhibit a broad range of protection, may be better suited than Mamu-A*01-postive monkeys for comparing vaccine efficacy in this model.

The addition of Ad5/gp140_jrfl to the Ad5/SIVGag vaccine tends toward better protection than the Ad5/SIVGag alone, and Ad5/gp140_jrfl alone was able to confer significant protection in the Mamu-A*01-negative monkeys. Ad5/gp140_jrfl was able to elicit significant cross-reactive CTL responses against 89.6P Env, which contributed to a broader protective CTL response. SHIV-89.6P-specific neutralizing antibody responses, in terms of both the level and timing after viral challenge, were largely indistinguishable between those cohorts receiving Ad5/gp140_jrfl, Ad5/SIVGag, and the combination of Ad5/gp140_jrfl and Ad5/SIVGag. The data collectively suggest that the protection conferred by the heterologous Env was due to elicitation of cross-reactive T-cell responses to Env rather than an antibody effect. This notion is consistent with the recent report by Letvin et al., who also found that a vectored heterologous Env (a BXB2/BaL chimera) vaccine conferred added protection by a mechanism mostly consistent with broadening virus-specific T-cell responses (21). Ad5/gp140_jrfl and Ad5/SIVGag have comparable efficacy in terms of protecting against the SHIV challenge. This finding that a heterologous Env antigen can be as efficacious as the well-studied viral internal antigen Gag suggests that Env antigen may serve as a component for a CTL-based HIV-1 vaccine.

It is important to note that the 80% sequence homology between JRFL gp140 and 89.6P gp140 is less that those exhibited by consensus amino acid sequences of Gag or Pol between different clades of HIV-1 viruses, which range from 84% to 92%. The fact that JRFL gp140 was able to elicit cross-reactive T-cell responses with apparent protective effect indicates that a vaccine composed of conserved viral genes such as Gag and Pol could potentially provide cross-clade T-cell responses and protection.

Amara et al. previously reported that elicitation of binding antibody by an Env component derived from HIV-1$_{89.6}$, the parental Env of SHIV-89.6P, was able to improve the efficacy of a Gag-Pol CTL vaccine against SHIV-89.6P (2). In this case, while immunization with the Env vaccine failed to elicit detectable neutralizing antibody responses prior to viral challenge, it could have potentially accelerated the development of neutralizing antibody responses following viral challenge. Willey et al. recently reported that using a pathogenic SHIV$_{DH12R-PS1}$ rhesus challenge model, a partially matched, parental Env component was able to prime for neutralizing antibody responses and confer protection against the pathogenic SHIV challenge (52). In the present study, we showed that among the Mamu-A*01-negative monkeys, the group receiving Ad5/gp140_89.6P exhibited the best disease attenuation. Two immunizations with high doses of Ad5/gp140_89.6P did not elicit detectable neutralizing antibody responses, yet did prime for a faster development of neutralizing antibodies after viral challenge. The results, in conjunction with those observed with partially matched Env, suggest that induction of neutralizing antibody responses, even at a suboptimal level, can significantly improve the CTL-based vaccine efficacy.

The Ad5/HIVTat vaccine elicited both T-cell and antibody responses, yet failed to control the viral replication or disease progression following the SHIV-89.6P challenge in the rhesus monkeys. Tat protein subunit vaccines, as well as vectored Tat vaccines, were previously shown to have activity against SHIV-89.6P infection in cynomolgus monkeys (5–7, 22). Pauza et al. showed that Tat toxoid vaccine was able to attenuate viral replication following intrarectal SHIV-89.6P challenge in rhesus monkeys (38). However, Silvera et al. reported that similar vaccines failed to protect against intravenous SHIV-89.6P challenge in rhesus monkeys (47). While the current study was in progress, Mooij et al. reported that a Tat vaccination with DNA priming plus protein boosting failed to protect against SHIV-89.6P challenge in rhesus monkeys (27). The Tat vaccines used in these studies were based on HIV-1 IIIB Tat sequence, whereas the Tat vaccine in the present study was based on the HIV-1jrfl Tat sequence. The sequence homology between the JRFL Tat and SHIV-89.6P Tat and that between IIIB Tat and SHIV-89.6P Tat are comparable, both around 88%. Because different vaccines and immunization and/or challenge regimens were used in the different studies, it is difficult to speculate on the underlining factor(s) responsible for the various outcomes among different studies. The lower SHIV-89.6P replication in cynomolgus monkeys could be in part responsible for the more reproducible protection observed in cynomolgus monkeys than that in rhesus monkeys. In this study, we compared a Tat vaccine antigen with other viral antigens using the same Ad5 vaccine vector, challenge viral stock, and similar immunization regimens. In this regard, we found that Tat was inferior to Gag or Env viral antigens in

terms of protection against SHIV-89.6 challenge in rhesus monkeys.

In summary, the studies above are informative in understanding the utility as well as the limitations of the SHIV challenge model in the development of an HIV vaccine. In particular, these experiments provide insight for understanding the relative protective potencies of selected virus antigens and the impact MHC alleles have on the efficacy of an immunization approach

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., J. M. Smith, S. I. Staprans, D. C. Montefiori, F. Villinger, J. D. Altman, S. P. O'Neil, N. L. Kozyr, Y. Xu, L. S. Wyatt, P. L. Earl, J. G. Herndon, J. M. McNicholl, H. M. McClure, B. Moss, and H. L. Robinson.** 2002. Critical role for Env as well as Gag-Pol in control of a simian-human immunodeficiency virus 89.6P challenge by a DNA prime/recombinant modified vaccinia virus Ankara vaccine. J. Virol. **76**:6138–6146.

3. **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.

4. **Burton, D. R., R. C. Desrosiers, R. W. Doms, W. C. Koff, P. D. Kwong, J. P. Moore, G. J. Nabel, J. Sodroski, I. A. Wilson, and R. T. Wyatt.** 2004. HIV vaccine design and the neutralizing antibody problem. Nat. Immunol. **5**:233–236.

5. **Cafaro, A., A. Caputo, C. Fracasso, M. T. Maggiorella, D. Goletti, S. Baroncelli, M. Pace, L. Sernicola, M. L. Koanga Mogtomo, M. Betti, A. Borsetti, R. Belli, L. Akerblom, F. Corrias, S. Butto, J. Heeney, P. Verani, F. Titti, and B. Ensoli.** 1999. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. Nat. Med. **5**:643–650.

6. **Cafaro, A., A. Caputo, S. Maggiorella, S. Baroncelli, C. Fracasso, M. Pace, A. Borsetti, L. Sernicola, D. R. Negri, P. Ten Haaft, M. Betti, Z. Michelini, I. Macchia, E. Fanales Belasio, R. Belli, F. Corrias, S. Butto, P. Verani, F. Titti, and B. Ensoli.** 2000. SHIV89.6P pathogenicity in cynomolgus monkeys and control of viral replication and disease onset by human immunodeficiency virus type 1 Tat vaccine. J. Med. Primatol. **29**:193–208.

7. **Cafaro, A., F. Titti, C. Fracasso, M. T. Maggiorella, S. Baroncelli, A. Caputo, D. Goletti, A. Borsetti, M. Pace, E. Fanales Belasio, B. Ridolfi, D. R. Negri, L. Sernicola, R. Belli, F. Corrias, I. Macchia, P. Leone, Z. Michelini, P. ten Haaft, S. Butto, P. Verani, and B. Ensoli.** 2001. Vaccination with DNA containing tat coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P). Vaccine **19**:2862–2877.

8. **Casimiro, D. R., L. Chen, T. M. Fu, R. K. Evans, M. J. Caulfield, M. E. Davies, A. Tang, M. Chen, L. Huang, V. Harris, D. C. Freed, K. A. Wilson, S. Dubey, D. M. Zhu, D. Nawrocki, H. Mach, R. Troutman, L. Isopi, D. Williams, W. Hurni, Z. Xu, J. G. Smith, S. Wang, X. Liu, L. Guan, R. Long, W. Trigona, J. Heidecker, H. C. Perry, N. Persaud, T. J. Toner, Q. Su, X. Liang, R. Youil, M. Chastain, A. J. Bett, D. B. Volkin, E. A. Emini, and J. W. Shiver.** 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 *gag* gene. J. Virol. **77**:6305–6313.

9. **Casimiro, D. R., A. Tang, L. Chen, T. M. Fu, R. K. Evans, M. E. Davies, D. C. Freed, W. Hurni, J. M. Aste Amezaga, L. Guan, R. Long, L. Huang, V. Harris, D. K. Nawrocki, H. Mach, R. D. Troutman, L. A. Isopi, K. K. Murthy, K. Rice, K. A. Wilson, D. B. Volkin, E. A. Emini, and J. W. Shiver.** 2003. Vaccine-induced immunity in baboons by using DNA and replication-incompetent adenovirus type 5 vectors expressing a human immunodeficiency virus type 1 *gag* gene. J. Virol. **77**:7663–7668.

10. **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.

11. **Condra, J. H., W. A. Schleif, O. M. Blahy, L. J. Gabryelski, D. J. Graham, J. C. Quintero, A. Rhodes, H. L. Robbins, E. Roth, M. Shivaprakash, and et al.** 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature **374**:569–571.

12. **Dale, C. J., R. De Rose, I. Stratov, S. Chea, D. C. Montefiori, S. Thomson,**

13. **I. A. Ramshaw, B. E. Coupar, D. B. Boyle, M. Law, and S. J. Kent.** 2004. Efficacy of DNA and fowlpox virus priming/boosting vaccines for simian/human immunodeficiency virus. J. Virol. **78**:13819–13828.

13. **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.

14. **Goulder, P. J., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael, and S. Rowland Jones.** 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat. Med. **3**:212–217.

15. **Goulder, P. J., Y. Tang, S. I. Pelton, and B. D. Walker.** 2000. HLA-B57-restricted cytotoxic T-lymphocyte activity in a single infected subject toward two optimal epitopes, one of which is entirely contained within the other. J. Virol. **74**:5291–5299.

16. **Hollander, M., and D. A. Wolfe.** 1999. Nonparametric statistical methods. 2nd ed. John Wiley & Sons, Inc., New York, N.Y.

17. **Johnson, P. R., B. C. Schnepp, M. J. Connell, D. Rohne, S. Robinson, G. R. Krivulka, C. I. Lord, R. Zinn, D. C. Montefiori, N. L. Letvin, and K. R. Clark.** 2005. Novel adeno-associated virus vector vaccine restricts replication of simian immunodeficiency virus in macaques. J. Virol. **79**:955–965.

18. **Kaslow, R. A., R. Duquesnoy, M. VanRaden, L. Kingsley, M. Marrari, H. Friedman, S. Su, A. J. Saah, R. Detels, J. Phair, et al.** 1990. A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection. A report from the Multicenter AIDS Cohort study. Lancet **335**:927–930.

19. **Kwong, P. D., M. L. Doyle, D. J. Casper, C. Cicala, S. A. Leavitt, S. Majeed, T. D. Steenbeke, M. Venturi, I. Chaiken, M. Fung, H. Katinger, P. W. Parren, J. Robinson, D. Van Ryk, L. Wang, D. R. Burton, E. Freire, R. Wyatt, J. Sodroski, W. A. Hendrickson, and J. Arthos.** 2002. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature **420**:678–682.

20. **Letvin, N. L.** 2005. Progress toward an HIV vaccine. Annu. Rev. Med. **56**:213–223.

21. **Letvin, N. L., Y. Huang, B. K. Chakrabarti, L. Xu, M. S. Seaman, K. Beaudry, B. Korioth Schmitz, F. Yu, D. Rohne, K. L. Martin, A. Miura, W. P. Kong, Z. Y. Yang, R. S. Gelman, O. G. Golubeva, D. C. Montefiori, J. R. Mascola, and G. J. Nabel.** 2004. Heterologous envelope immunogens contribute to AIDS vaccine protection in rhesus monkeys. J. Virol. **78**:7490–7497.

22. **Maggiorella, M. T., S. Baroncelli, Z. Michelini, E. Fanales Belasio, S. Moretti, L. Sernicola, A. Cara, D. R. Negri, S. Butto, V. Fiorelli, A. Tripiciano, A. Scoglio, A. Caputo, A. Borsetti, B. Ridolfi, R. Bona, P. ten Haaft, I. Macchia, P. Leone, M. R. Pavone Cossut, F. Nappi, M. Ciccozzi, J. Heeney, F. Titti, A. Cafaro, and B. Ensoli.** 2004. Long-term protection against SHIV89.6P replication in HIV-1 Tat vaccinated cynomolgus monkeys. Vaccine **22**:3258–3269.

23. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12. Virology **36**:115–125.

24. **Mascola, J. R.** 2002. Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. Vaccine **20**:1922–1925.

25. **Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx, and M. G. Lewis.** 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. **6**:207–210.

26. **Miگueles, S. A., M. S. Sabbaghian, W. L. Shupert, M. P. Bettinotti, F. M. Marincola, L. Martino, C. W. Hallahan, S. M. Selig, D. Schwartz, J. Sullivan, and M. Connors.** 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc. Natl. Acad. Sci. USA **97**:2709–2714.

27. **Mooij, P., I. G. Nieuwenhuis, C. J. Knoop, R. W. Doms, W. M. Bogers, P. J. Ten Haaft, H. Niphuis, W. Koornstra, K. Bieler, J. Kostler, B. Morein, A. Cafaro, B. Ensoli, R. Wagner, and J. L. Heeney.** 2004. Qualitative T-helper responses to multiple viral antigens correlate with vaccine-induced immunity to simian/human immunodeficiency virus infection. J. Virol. **78**:3333–3342.

28. **Mothe, B. R., J. Weinfurter, C. Wang, W. Rehrauer, N. Wilson, T. M. Allen, D. B. Allison, and D. I. Watkins.** 2003. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J. Virol. **77**:2736–2740.

29. **Muhl, T., M. Krawczak, P. Ten Haaft, G. Hunsmann, and U. Sauermann.** 2002. MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys. J. Immunol. **169**:3438–3446.

30. **Nabel, G. J.** 2003. Vaccine for AIDS and Ebola virus infection. Virus Res. **92**:213–217.

31. **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.

32. **Nishimura, Y., T. Igarashi, N. L. Haigwood, R. Sadjadpour, O. K. Donau, C. Buckler, R. J. Plishka, A. Buckler White, and M. A. Martin.** 2003. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. Proc. Natl. Acad. Sci. USA **100:**15131–15136.

33. **O'Connor, D. H., B. R. Mothe, J. T. Weinfurter, S. Fuenger, W. M. Rehrauer, P. Jing, R. R. Rudersdorf, M. E. Liebl, K. Krebs, J. Vasquez, E. Dodds, J. Loffredo, S. Martin, A. B. McDermott, T. M. Allen, C. Wang, G. G. Doxiadis, D. C. Montefiori, A. Hughes, D. R. Burton, D. B. Allison, S. M. Wolinsky, R. Bontrop, L. J. Picker, and D. I. Watkins.** 2003. Major histocompatibility complex class I alleles associated with slow simian immunodeficiency virus disease progression bind epitopes recognized by dominant acute-phase cytotoxic-T-lymphocyte responses. J. Virol. **77:**9029–9040.

34. **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.

35. **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 SIVmac-induced immunodeficiency. J. Virol. **76:**292–302.

36. **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.

37. **Patterson, L. J., N. Malkevitch, D. Venzon, J. Pinczewski, V. R. Gomez Roman, L. Wang, V. S. Kalyanaraman, P. D. Markham, F. A. Robey, and M. Robert Guroff.** 2004. Protection against mucosal simian immunodeficiency virus SIV_{mac251}) challenge by using replicating adenovirus-SIV multigene vaccine priming and subunit boosting. J. Virol. **78:**2212–2221.

38. **Pauza, C. D., P. Trivedi, M. Wallace, T. J. Ruckwardt, H. Le Buanec, W. Lu, B. Bizzini, A. Burny, D. Zagury, and R. C. Gallo.** 2000. Vaccination with tat toxoid attenuates disease in simian/HIV-challenged macaques. Proc. Natl. Acad. Sci. USA **97:**3515–3519.

39. **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.

40. **Richman, D. D., T. Wrin, S. J. Little, and C. J. Petropoulos.** 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc. Natl. Acad. Sci. USA **100:**4144–4149.

41. **Robinson, H. L., D. C. Montefiori, R. P. Johnson, K. H. Manson, M. L. Kalish, J. D. Lifson, T. A. Rizvi, S. Lu, S. L. Hu, G. P. Mazzara, D. L. Panicali, J. G. Herndon, R. Glickman, M. A. Candido, S. L. Lydy, M. S. Wyand, and H. M. McClure.** 1999. Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. Nat. Med. **5:**526–534.

42. **Ruprecht, R. M., F. Ferrantelli, M. Kitabwalla, W. Xu, and H. M. McClure.** 2003. Antibody protection: passive immunization of neonates against oral AIDS virus challenge. Vaccine **21:**3370–3373.

43. **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.

44. **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.

45. **Shiver, J. W., and E. A. Emini.** 2004. Recent advances in the development of HIV-1 vaccines using replication-incompetent adenovirus vectors. Annu. Rev. Med. **55:**355–372.

46. **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.

47. **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.

48. **Tang, Y., F. Villinger, S. I. Staprans, R. R. Amara, J. M. Smith, J. G. Herndon, and H. L. Robinson.** 2002. Slowly declining levels of viral RNA and DNA in DNA/recombinant modified vaccinia virus Ankara-vaccinated macaques with controlled simian-human immunodeficiency virus SHIV-89.6P challenges. J. Virol. **76:**10147–10154.

49. **van Baalen, C. A., O. Pontesilli, R. C. Huisman, A. M. Geretti, M. R. Klein, F. de Wolf, F. Miedema, R. A. Gruters, and A. D. Osterhaus.** 1997. Hum. immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. J. Gen. Virol. **78:**1913–1918.

50. **VaxGen.** 24 February 2003. VaxGen announces initial results of its Phase III AIDS vaccine trial. www.vaxgen.com/pressroom/index.html.

51. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, J. F. Salazar Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. Nature **422:**307–312.

52. **Willey, R. L., R. Byrum, M. Piatak, Y. B. Kim, M. W. Cho, J. L. Rossio, Jr., J. Bess, Jr., T. Igarashi, Y. Endo, L. O. Arthur, J. D. Lifson, and M. A. Martin.** 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. J. Virol. **77:**1163–1174.

53. **Xu, W., R. Hofmann Lehmann, H. M. McClure, and R. M. Ruprecht.** 2002. Passive immunization with human neutralizing monoclonal antibodies: correlates of protective immunity against HIV. Vaccine **20:**1956–1960.

54. **Zagury, J. F., A. Sill, W. Blattner, A. Lachgar, H. Le Buanec, M. Richardson, J. Rappaport, H. Hendel, B. Bizzini, A. Gringeri, M. Carcagno, M. Criscuolo, A. Burny, R. C. Gallo, and D. Zagury.** 1998. Antibodies to the HIV-1 Tat protein correlated with nonprogression to AIDS: a rationale for the use of Tat toxoid as an HIV-1 vaccine. J. Hum Virol. **1:**282–292.

55. **Zhang, Z. Q., T. M. Fu, D. R. Casimiro, M. E. Davies, X. Liang, W. A. Schleif, L. Handt, L. Tussey, M. Chen, A. Tang, K. A. Wilson, W. L. Trigona, D. C. Freed, C. Y. Tan, M. Horton, E. A. Emini, and J. W. Shiver.** 2002. Mamu-A*01 allele-mediated attenuation of disease progression in simian-human immunodeficiency virus infection. J. Virol. **76:**12845–12854.